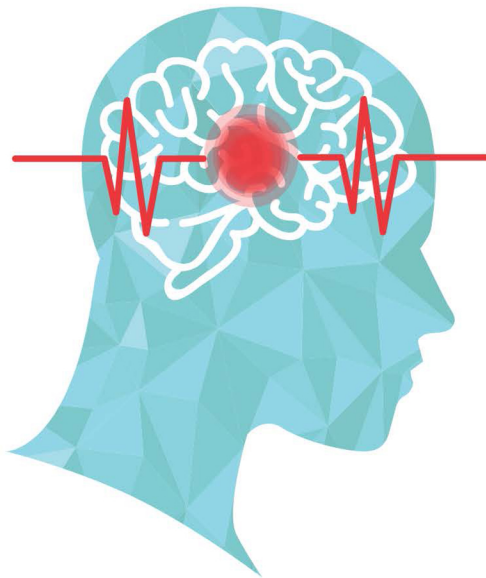


NEW DEVELOPMENTS IN MEDICAL RESEARCH

Encyclopedia of Cerebral Vascular Accidents

(9 Volume Set)



VOLUME
1

Sean M. Townsend • John M. Horst
Editors

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NEW DEVELOPMENTS IN MEDICAL RESEARCH

**ENCYCLOPEDIA OF CEREBRAL
VASCULAR ACCIDENTS
VOLUME 1
(9 VOLUME SET)**

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(9 VOLUME SET)

**SEAN M. TOWNSEND
AND
JOHN M. HORST
EDITORS**



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PREFACE

This 9 volume set covers a wide range of topics, including:

- childhood vasculitic stroke
- anosognosia
- stroke-rehabilitation games
- electrical stimulation
- vasculitis

Chapter 1

MANAGEMENT OF CAROTID STENOSIS: CAROTID REVASCULARIZATION IN THE MODERN ERA

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ABSTRACT

Carotid artery atherosclerosis is responsible for 10-15% of all supratentorial ischemic strokes. Over the last 2 decades, several clinical trials for carotid revascularization have been conducted, which have influenced our choices of revascularization procedures. Carotid artery stenting, previously regarded as an option for high surgical risk patients only, is now proposed as a reasonably effective and safe alternative to carotid endarterectomy for certain classes of young subjects. The treatment of asymptomatic carotid stenosis is still under debate. Evidence is accumulating that improved medical therapy has led to a lower risk of stroke in medically treated patients with asymptomatic carotid stenosis, than the periprocedural risk from revascularization therapies.

In this review, we will examine carotid revascularization from a historical perspective. We will review data from contemporary clinical trials that have shaped contemporary understanding of the efficacy and safety of Carotid artery Stenting and Endarterectomy. Finally, we will review the evidence supporting intensive medical therapy for asymptomatic patients with carotid stenosis.

ATHEROSCLEROSIS: THE ROLE OF INFLAMMATION

Atherosclerosis, as we currently understand it, is an inflammatory disease. It is a multifocal process, simultaneously affecting various medium and large arterial beds such as

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the coronary, cerebral and peripheral arterial vasculature [1]. The endothelium plays a dynamic role in the inflammatory process. Leucocytes and intimal smooth muscle are also actively involved. Inflammation plays a key role, in the initiation, progression and vulnerability of atherosclerotic plaque [1].

Normal endothelium is quiescent. It is in an anti-inflammatory state, with excess production of nitric oxide: regarded protective for the endothelium. Vascular risk factors such as hypertension, diabetes, oxidized low density lipoprotein (LDL) cholesterol, very low density lipoprotein (VLDL) cholesterol, smoking; homocysteine; certain infections and in case of the extracranial carotid artery, mechanical shear stresses in the region of the carotid bulb, convert the anti-inflammatory endothelial cell into a pro-inflammatory state [1]. This is characterized by excess production of oxygen-derived free radicals such as superoxide and peroxynitrites. One of the major effects of oxygen-derived free radicals is the oxidation of LDL cholesterol. Oxidized LDL has a major role in the initiation and propagation of atherosclerotic plaque. It results in activation of other endothelial cells as mentioned above. It induces production of endothelium adhesion molecules, which attract monocytes to the area. Further, it activates monocytes into macrophages, which engulf oxidized LDL molecules resulting in foamy macrophages. Oxidized LDL also reduces the expression of nitric oxide synthase, decreasing the production of protective nitric oxide [1]. Another downstream effect of oxidative stress is the expression of nuclear factor-KappaB (NF-KB). This molecule also results in expression of endothelial cell adhesion molecules, which attract inflammatory cells. It also results in increased expression of matrix metalloproteinase 9: MMP 9 (a molecule that has received considerable interest in the recent years in the field of coronary and cerebral atherosclerosis). The molecule results in deterioration of the extracellular matrix; promoting migration of leukocytes and smooth muscle cells into the sub endothelial area. In the fully developed plaque, MMP 9 may weaken the fibrin cap, resulting in plaque rupture [1].

The American Heart Association has described various stages of atherosclerotic plaque evolution, starting with an initial lesion when macrophages are activated [2]. This progresses to a fatty streak where foamy macrophages are formed. These progress to intermediate lesions where foamy macrophages increase in number, and some of them die resulting in extracellular lipid formation [2]. Up to this stage, plaques are usually asymptomatic. As the extracellular lipid increases in quantity, the plaque is now called an atheroma. This progresses to a fibroatheroma with a defined lipid necrotic core and a fibrous cap [2]. When sufficient amount of necrotic lipid accumulates, it may crystallize into cholesterol crystals [3]. The jagged crystals may cause a rupture of the fibrous cap or may rupture the vasa vasorum of the artery resulting in intraplaque hemorrhage. Such a complicated lesion is the setting for initiation of thrombosis [3]. Once a necrotic lipid core begins to form, the patient is prone to develop clinical symptoms.

EVOLUTION OF CAROTID REVASCLARIZATION

In 1954, a case report of the removal of the stenotic segment of the carotid artery was published [4]. Over the next couple of decades, carotid endarterectomy (CEA) evolved as a method of treatment for carotid stenosis. Up to 1985, two negative randomized trials comparing CEA with medical therapy were published [5, 6]. In spite of negative trials, the

utilization of CEA procedures continued worldwide. Around this time, the EC-IC (External Carotid- Internal Carotid) bypass study for treatment of carotid stenosis was conducted, and the study failed to show benefit [7]. It was in this setting that the NASCET (The North American Symptomatic Carotid Endarterectomy trial) was conceived and eventually began randomization in 1987 [8].

While there have been numerous clinical trials and registries that have shaped our understanding of the treatment of carotid stenosis over the last 25 years, in this discussion, we will concentrate on trials conducted in the United States and those that have provided the most important information.

THE NORTH AMERICAN SYMPTOMATIC CAROTID ENDARTERECTOMY TRIAL (NASCET)

The North American Symptomatic Carotid Endarterectomy trial recruited patients with symptomatic carotid stenosis greater than 30%, and randomized them to medical therapy versus CEA [8]. By 1991, the benefit of the procedure in patients with severe stenosis of 70 to 99% was established [9]. Thereafter, up to the end of recruitment in 1996, only patients with moderate stenosis were randomized. The perioperative stroke and death rate (up to 30 days) in this trial was 6.7% [8]. The average follow-up was 5 years. If patients in the medical arm progressed to greater than 70% stenosis, they were offered CEA.

After an average 24 months follow-up, patients with severe carotid stenosis of 70 to 99% showed a dramatic risk reduction of any ipsilateral stroke from 26% in the medical arm to 9% in the CEA arm. The absolute risk reduction was 17% ($p < 0.001$), which translated to a robust number needed to treat of 6. Among patients with moderate stenosis of 50 to 69%, CEA showed a significant but less robust reduction in risk of any ipsilateral stroke from 22.2% in the medical arm to 15.7% in the CEA arm. The absolute risk reduction was 6.5% ($p = 0.045$), giving a number needed to treat of 15 [8]. Patients with mild stenosis of 30 to 49% did not achieve a significant risk reduction of any ipsilateral stroke following CEA (medical arm 18.7% versus CEA arm 14.9%; absolute risk reduction 3.8% ($p = 0.16$) [8].

The NASCET trial, which completed randomization in 1996, set the paradigm for treatment of patients with moderate to severe symptomatic carotid stenosis. These patients are always treated with a revascularization procedure. Till date, no clinical trials after NASCET have challenged the paradigm of revascularization of symptomatic carotid stenosis. This is in spite of the fact that the comparison medical arm in NASCET would be considered far from adequate in this day and age. Only 16% of patients assigned to the medical arm and 13% of patients assigned to the CEA arm were on lipid lowering medications [8].

IDEAL TIMING FOR REVASCULARIZATION PROCEDURES

Following an ischemic stroke, the highest risk for recurrent stroke is during the first month; the risk decreases with time due to plaque stabilization and the development of collaterals [10]. Presumably, then, the benefit obtained from carotid revascularization for a symptomatic carotid stenosis would decline with time. On the other hand, very early

revascularization procedures could potentially lead to hemodynamic changes within an acutely necrotic area of infarcted brain, resulting in intracerebral hemorrhage [10].

To answer this question, Rothwell et al. reviewed patient data from the NASCET trial and a similar European clinical trial: The European Carotid Surgery Trial (ECST) [11]. This included 5893 patients with 33,000 patient years of follow-up. They reviewed a primary outcome of any stroke or death within 30 days and five-year ipsilateral stroke. Patients with moderate stenosis and severe stenosis showed significant benefits from CEA if the procedure was performed within 2 weeks of the index event. The benefit declined thereafter, and was no longer statistically significant beyond 2 weeks [11]. Based on this data, the American Heart Association recommends that it is reasonable to perform CEA when indicated for patients with stroke or TIA within 2 weeks, rather than delaying surgery [12]. Some contraindications to surgery within 2 weeks could be patients who have very large infarctions at high risk of hemorrhagic conversion, or already have hemorrhagic conversion of their infarctions. In these scenarios it may be prudent to wait beyond 2 weeks.

THE ASYMPTOMATIC CAROTID ARTERY SURGERY TRIAL (ACAS)

The Asymptomatic Carotid Artery Surgery trial recruited patients between 1987 and 1993. The study randomized 1662 asymptomatic patients up to 79 years of age with greater than 60% stenosis to CEA versus medical therapy [13]. In this study, the perioperative stroke and death rate (30 days) was 2.3%. The risk of perioperative stroke and death, and five-year risk of ipsilateral stroke was 11% in the medical arm (the annual risk of ipsilateral stroke in the medical arm was 2.3%) and 5.1% in the CEA arm (p value=0.004) [13]. Thus, there was an absolute risk reduction of 5.9% for the primary outcome by offering CEA. Upon further review of the Kaplan-Meier curves in the study, the CEA curve crossed the medical arm 10 months out from the procedure and a statistically significant benefit of CEA over medical therapy was only seen after 3 years [13]. Thus, in the asymptomatic population the benefit of CEA was less robust and more delayed than noted in the NASCET trial with symptomatic patients.

There were some additional findings of interest in the ACAS study. While the overall relative risk reduction with CEA over medical therapy for asymptomatic carotid stenosis was 57%, there was a sex difference favoring males. The relative risk reduction in males was 66% versus a less impressive 17% among females with asymptomatic stenosis [13]. The benefit of revascularization was greater amongst younger patients less than 68 years: relative risk reduction of 60% versus 43% in patients older than 68 years [13].

THE ASYMPTOMATIC CAROTID SURGERY TRIAL (ACST-1)

A more contemporary trial on patients with asymptomatic carotid stenosis was the Asymptomatic Carotid Surgery trial. Patients were recruited between 1993 and 2003 [14]. In all, 3120 asymptomatic patients were randomized to immediate CEA versus indefinite deferral of CEA (medical therapy). The median follow-up was 9 years, providing an excellent opportunity to study the durability of the results of CEA. In this study, the perioperative risk

of stroke or death was 3%. The study found a significant protective effect of CEA against any stroke or perioperative death at 5 years (10.9% in the deferred arm versus 6.9% in the immediate CEA arm, $p=0.0001$) [14]. This benefit was sustained up to 10 years (17.9% in the deferred arm versus 13.4% in the immediate CEA arm, $p=0.009$) [14].

While the ACST 1 trial showed a durable benefit of CEA in asymptomatic patients, there are certain caveats worthy of note. The intensity of medical therapy evolved significantly over the duration of the trial. The use of antihypertensive therapy improved from 55% to 87% in the medical arm and 51% to 89% in the CEA arm over the 10 years of the study, leading to a decrease in mean diastolic pressures of 6.5 mmHg over the course of the trial [14]. The use of lipid lowering therapies improved from 11% at the start of the trial to 80% by the end of the study in the medical arm and from 7% to 82% in the CEA arm [14]. As the quality and intensity of medical therapy improves over the years, it has become increasingly difficult to justify the use of a revascularization procedure in the asymptomatic population.

STENTING AND ANGIOPLASTY WITH PROTECTION IN PATIENTS AT HIGH RISK FOR ENDARTERECTOMY TRIAL (SAPPHIRE)

The turn of the century saw increasing utilization of angioplasty and carotid artery stenting (CAS) as a carotid revascularization technique. This method provided an attractive alternative for revascularization in the patient who was at high surgical risk. In this setting, Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy trial was designed. Between 2000 and 2002, 334 patients (greater than 50% stenosis if symptomatic and greater than 80% stenosis if asymptomatic) were randomized to CEA versus CAS with embolic protection device [15]. The study defined high-risk surgical criteria which included clinically significant cardiac disease (congestive heart failure, abnormal stress test, need for open-heart surgery), severe lung disease, contralateral carotid occlusion, contralateral laryngeal nerve palsy, previous radical neck surgery, previous radiation to the neck, recurrent stenosis after CEA and age greater than 80 years. The trial was powered for noninferiority rather than superiority [15].

The primary outcome of 30 day death, stroke and myocardial infarction (MI) and one year stroke or neurological death was observed in 12.2% of the CAS arm and 20.1% of the CEA arm [15]. This did not reach statistical significance for superiority, but was statistically significant for noninferiority ($p=0.004$). The secondary outcomes: major ipsilateral stroke (0.6% in the CAS arm and 3.3% in the endarterectomy arm), minor ipsilateral stroke (3.7% in the CAS arm and 2.0% in the CEA arm), MI (3.0% with CAS and 7.5% with CEA) were not significantly different in the intention to treat population. However, in the per protocol population, the CAS arm had significantly fewer major strokes (0% versus 3.5%, $p=0.02$) and significantly fewer MIs (2.5% versus 8.1% with CEA, $p=0.03$) [15]. While the primary outcome was not significantly different in the symptomatic subgroup of patients (16.8% with CAS and 16.5% with CEA, $p=0.95$); CAS proved significantly safer with regards to the primary outcome among the asymptomatic subgroup (9.9% with CAS and 21.5% with CEA, $p=0.02$). The study concluded that CAS was noninferior to CEA for high-risk populations for prevention of stroke, death or MI [15].

THE CAROTID REVASCULARIZATION ENDARTERECTOMY VERSUS STENTING TRIAL (CREST)

The studies described in the previous sections laid the ground for the Carotid Revascularization Endarterectomy versus Stenting trial. Most of our current understanding regarding the management of carotid stenosis is based on the findings of this trial. The trial started recruiting symptomatic patients in 2001 [16]. In 2005, recruitment was extended to asymptomatic patients in order to reflect the real-world scenario, where the majority of revascularization procedures are performed amongst asymptomatic patients. Inclusion of asymptomatic patients markedly improved the recruitment rate. The primary outcome was stroke, MI or death during the periprocedural period or any ipsilateral stroke within 4 years.

Interventionalists participating in CREST underwent a rigorous certification procedure. Physicians who did not meet the stringent requirements in terms of experience and complication rates were entered in a “lead in” phase [16]. Physicians were allowed to enter the randomization phase of the trial only after they had successfully completed the lead-in phase. Another feature was the use of predetermined stents and embolus protection devices as well as periprocedural medication dosages. A noteworthy feature about the assessment of outcomes was the regular screening for MIs with EKGs and cardiac enzymes before and after the procedure [16]. Some critics question the inclusion of MI within the primary outcome while evaluating procedures intended for stroke prevention [17].

At the end of 4 years of follow-up, the primary outcome occurred in 7.2% of the 1262 patients in the CAS arm and 6.8% of the 1240 CEA patients, $p=0.51$ [16]. Evaluation of Kaplan-Meier curves suggested no difference in the primary outcome between the procedures throughout the duration of follow-up. However, upon review of the individual components of the outcome, certain significant differences were observed. When compared with the CEA arm, patients in the CAS arm had significantly higher perioperative strokes (4.1% CAS versus 2.3% CEA, $p=0.01$) and perioperative minor ipsilateral strokes (2.9% CAS versus 1.4% CEA, $p=0.009$) [16]. Perioperative MI was significantly higher among the CEA patients (1.1% CAS arm versus 2.3% CEA, $p=0.03$). The significant increase in strokes among the CAS patients was noted up to 4 years (6.2% CAS versus 4.7% CEA, $p=0.049$) [16]. The traditionally accepted outcome of stroke and death in the perioperative period and stroke up to 4 years of follow-up was also significantly higher in the CAS arm (6.4% with CAS and 4.7% with CEA, $p=0.03$) [16]. It appeared that the increased rate of strokes seen with CAS was balanced by increased periprocedural MI with CEA.

As outcomes go, do strokes or MIs have greater impact? The physical component of the SF 36 questionnaire was significantly worse at one year among stroke patients, but showed an uncertain effect among MI patients. The mental component was also significantly worse among stroke patients at one year [16]. On the other hand, mortality rates were more than 3 times higher among patients who suffered an MI in the perioperative period, and also amongst patients who only had elevations of cardiac enzymes. This was true even after adjustment of baseline co-morbid factors. [18]

CAROTID REVASCULARIZATION IN ASYMPTOMATIC PATIENTS

In the CREST study, about 47% of the overall population was asymptomatic [16]. The rate of the primary outcome was similar: 5.6% in the CAS arm and 4.9% in the CEA arm [19]. None of the individual outcomes were significantly different in the asymptomatic subset. It is noteworthy that the stroke and death rate by 4 years was 4.5% in the CAS arm and 2.7% in the endarterectomy arm [19]. While not statistically significant, this difference may be clinically important. CREST was not designed for asymptomatic patients initially. The study methods did not provide enough power to identify significant differences in the asymptomatic subgroup. It is plausible that a similar trial designed with sufficient power in the asymptomatic subgroup would have found the above difference in stroke and death rate to be significant [17].

CAROTID REVASCULARIZATION IN THE ELDERLY

An important finding in the CREST study was an effect modification of the primary outcome by age. Patients older than 70 years of age appeared to do better with CEA, whereas patients younger than 70 years of age fared better with CAS ($p=0.02$ for interaction) [16]. The rate of primary outcome with CEA was 6.1% for subjects younger than 65 years, 6.8% for subjects between 65 to 74 years and 7.4% for subjects 75 years and older [20]. On the other hand, there was a progressive rise in the rate of primary outcome in the CAS arm with age: 3.9% if less than 65 years, 6.3% in the 65 to 74 years age group and 12.7% in patients 75 years and older [20]. Symptomatic status did not modify this effect further i.e., the age-treatment interaction was found in symptomatic and asymptomatic subjects [20].

A similar interaction was also found when the stroke endpoint (periprocedural stroke and post procedure ipsilateral stroke) was examined: 3.7% in patients younger than 65, 5.1% in subjects 65 to 74 years and 10.9% in subject 75 years and older ($p=0.033$ for interaction) [20]. If perioperative stroke and ipsilateral stroke up to 4 years was considered, patients in the CAS arm fared worse than CEA patients if they were over the age of 64 years [20].

The interaction with age was also seen in a meta-analysis of 3 carotid intervention trials: EVA-3S, SPACE and ICSS [21]. The event rate for any stroke or death in the CEA arm was 5.7% in subjects younger than 70 years and 5.9% in subjects 70 years and older. On the other hand, any stroke or death occurred in 5.8% of subjects younger than 70 years in the CAS arm and 12% of subjects 70 years and older [21].

CAPTURE2 is a carotid stent post-marketing registry that recently published results [22]. Out of 5297 patients in the registry 1166 subjects were over the age of 80; only 15% of this group was symptomatic [22]. In other words, close to a fifth of the patients in this registry were elderly and asymptomatic: a demographic with hardly any clinical trial evidence. The 30-day stroke and death rate in the subgroup of patients over 80 years was 4.5% [22]. In the CREST lead in phase, 432/1565 subjects were 75 years and older. The overall 30-day stroke and death rate in subjects over 75 years was 7.5% (5.4% in the asymptomatic group [23]). SAPHIRE WW is another post marketing registry where 520 out of the reported 2001 patients were over the age of 80 years. These elderly patients had nearly twice the rate of 30-day stroke and death compared to patients below the age of 80 years (OR: 1.92) [24]. While

these data suggest that CAS in the elderly have high rates of 30-day stroke and death; we have very little clinical trial data of complication rates from CEA or medical therapy in this subgroup. The ACST-1 study had 30/1662 patients in the CEA arm who were over the age of 75 years, and 38/1701 subjects in the medical arm over the age of 75 years [14]. Within this subgroup of elderly patients in ACST 1, medical therapy achieved equivalent results to revascularization by CEA (OR: 0.81, 95% CI 0.43-1.51) [14].

CAROTID REVASCULARIZATION IN WOMEN

Across most carotid revascularization trials, women tend to carry more perioperative risk than men. The smaller diameter of the carotid artery in women compared to men is the presumed cause for this phenomenon [25]. Gender wise differences in perioperative risk from carotid revascularization in the CREST study have been published. The rate of 30-day stroke, death or MI in the CAS arm was 4.3% amongst men and 6.8% amongst women [26]. This complication rate with CAS was not significantly different than CEA in men (4.9%); but the rate in women was significantly higher in the CAS arm compared with the CEA arm (3.8%, $p=0.047$) [26]. Among women, the 30-day stroke and death rate in the CAS group was 5.5%, and 2.2% in the CEA group, $p=0.013$ [26]. CAS amongst the symptomatic women also resulted in a significantly higher 30-day stroke and death rate compared to CEA (7.5% versus 2.7%, $p=0.03$). In the asymptomatic women, CAS had a higher, albeit nonsignificant rate of 30-day stroke and death compared to endarterectomy (3.3% versus 1.6%, $p=0.28$) [26].

The author reviewed contemporary in-hospital stroke and death rates following carotid CAS within his own institution. Between June 2009 and May 2011, 483 carotid revascularizations were performed, out of which 307 (63.6%) were carotid stents. The in-hospital stroke and death rate was higher amongst women than men (4.4% versus 1.6%). In asymptomatic women, the in-hospital stroke and death rate was 4.5%-note that the nationwide benchmark for 30-day stroke and death rate is less than 3%. In the subset of women who were asymptomatic and over the age of 70 years, the in-hospital stroke and death rate was 6.1%.

PHYSICIAN SPECIALTY AND THE CHOICE OF REVASCULARIZATION PROCEDURE

At the author's own institution, review of contemporary data revealed that 87% of CAS were performed by cardiologists [27]. In spite of the age interaction demonstrated in CREST, where patients older than 70 years fared worse with CAS, 50.3% of patients receiving carotid artery stents after the announcement of the CREST results were 70 years old and above. In fact, following CREST, a significant increase in the proportion of procedures performed for nonspecific neurological symptoms such as lightheadedness or dizziness was noted (5.5% of procedures before CREST versus 18.3% of procedures after CREST, $p=0.002$) [27]. Presumably, the elderly are more likely to have nonspecific symptoms, for which they may receive a carotid duplex screen by their internists. Detection of a carotid stenosis in this manner may prompt a cardiology referral for CAS, as internists may believe it to be a less invasive procedure. Elderly patients are also more likely to have cardiac disease, and be

evaluated by cardiologists, who may screen the carotid vessels during evaluation of the coronary vasculature, picking up asymptomatic stenoses.

These theories of the role of physician specialty in the selection of carotid revascularization procedures were tested in a study on Medicare beneficiaries between 2005 and 2007 [28]. Among all specialties performing CAS, utilization of the procedure increased the most among cardiologists. Cardiologists comprised one third of all operators, but performed 52% of all carotid stents [28]. The patients who received stents from cardiologists were more likely to have higher rates of invasive cardiac procedures and were more likely to be asymptomatic (bearing out the aforementioned theories). In this study, 30-day outcomes did not vary by specialty [28].

CAROTID CAS COMPLICATION RATES OVER TIME

As technology advances, improvements in techniques and skill may result in lower perioperative complication rates with CAS. Published data from carotid artery standing post-marketing registries over the last decade appear to suggest this phenomenon [29]. Within the CREST study however, the perioperative stroke and death rates remained steady between 2000 and 2008 [30]. Over time, in CREST, the proportion of asymptomatic patients progressively increased and the proportion of elderly patients over the age of 80 years progressively decreased. Even after adjustment for symptomatic status and age, the perioperative stroke and death rates did not change over time in CREST [30].

A similar finding was demonstrated in the analysis of in-hospital complications following CAS in the Nationwide Inpatient Sample [31]. The trend of in-hospital stroke, death or MI did not change between 2004 and 2009. Trends were examined for asymptomatic and symptomatic patients, males and females, elderly patients 80 years and older as well as younger patients below the age of 80 years. Complication rates did not trend downward in any of these subgroups.

MAKING THE CASE FOR INTENSIVE MEDICAL THERAPY FOR ASYMPTOMATIC CAROTID STENOSIS

Spence et al. have demonstrated the efficacy of a new treatment paradigm instituted in their population since 2003 [32]. In this paradigm, carotid plaque area is monitored. Medical therapy including lifestyle changes, lipid management, blood pressure control and use of medications for insulin resistance is progressively intensified upon increases in plaque area. Using such intense medical therapy, they were able to significantly lower the rates of stroke, death and MI. The authors in that study also followed patients with transcranial Doppler monitoring for micro emboli. They demonstrated a significant drop in percentage of patients with micro emboli following the institution of the new paradigm of intensive medical therapy [32]. The authors also demonstrated a significantly higher rate of stroke, death and MI among patients with micro emboli in comparison with those without micro emboli [32]. Transcranial Doppler may prove to be an important tool to identify patients with asymptomatic carotid

stenosis who are at a higher risk of vascular events. In other words, micro emboli may serve as a useful biomarker of vulnerable carotid plaque.

Anne Abbott reported a meta-analysis of 11 asymptomatic carotid intervention studies between 1985 and 2007 [33]. She used raw data from these trials to calculate rates of ipsilateral stroke, ipsilateral stroke/TIA, any territory stroke and any territory stroke/TIA in the medical therapy arm. There was a significant reduction in the rate of each outcome over the years [33]. The outcome rates in the more contemporary studies were quite similar, if not better than the CEA arm in the ACAS trial [33]. Considering the extremely low stroke rate achieved by intensive medical therapy among patients with asymptomatic carotid stenosis, the benchmark of 3% perioperative stroke or death from revascularization procedures set by the American Heart Association seems generous and may have to be reduced further.

FUTURE DIRECTIONS

Two clinical trials are currently randomizing patients with asymptomatic carotid stenosis to CAS versus CEA. ACT-1 (the Asymptomatic Carotid trial) is randomizing asymptomatic patients at standard risk for surgery. CAS procedures in this class of patients are not reimbursed currently in the United States. The ACST-2 (Asymptomatic Carotid Surgery trial-2) is randomizing standard and high risk patients with asymptomatic stenosis to CEA versus CAS. In view of the extremely low event rates noted with contemporary medical therapy among asymptomatic patients, a carotid intervention study with a medical arm for asymptomatic patients is being planned and currently awaits funding.

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Chapter 2

ISCHEMIC STROKE: FROM ACUTE TREATMENT TO LONG-TERM RECOVERY

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ABSTRACT

Ischemic stroke (IS) is due to the sudden occlusion of a cerebral artery with the consequence of a critical reduction in cerebral blood flow in a localized region (rCBF) of the brain. It represents the second cause of death and the leading cause of neurological disability in developed countries. Primarily due to the resulting economic burden, IS is considered a world-wide challenge. Different strategies can be identified to face this challenge: acute treatment, primary and secondary prevention, rehabilitation.

For several years acute IS treatment has been based on the use of the only current drug approved in the first 3 hours from symptom onset: recombinant tissue plasminogen activator (rtPA). However, mainly due to the short therapeutic window, a low percentage of patients can be actually treated by rtPA. Thus, with improving diffusion of more accurate diagnostic tools and of prognostic informations in different clinical scenarios, new treatment strategies in the acute phase have been developed such as intra-arterial and mechanical thrombolysis.

Prevention plays a crucial role in counteracting the economic burden of IS through life-style changes, vascular risk factors control (with arterial hypertension being the main one for IS) and antiplatelet therapy. Moreover prevention therapy has been enriched by the introduction, in the last few years, of new drugs with pleiotropic effects such as statins and ACE-inhibitors. Also potential pleiotropic effects of antidiabetic drugs are currently under investigation. New insights in secondary prevention come from the use of phosphodiesterase inhibitors such as dipyridamole (in combination with aspirine) and cilostazol. The latter demonstrated to be more effective than aspirin in reducing the risk

of recurrent stroke with the advantage of dramatically reducing the risk of haemorrhagic events as compared to aspirin.

Rehabilitation strategies are weighted, together with acute IS treatment, by most of stroke physicians and patients expectations. Among them transcranial magnetic stimulation (TMS) is a new tool able to explore and affect critical aspects of neural plasticity, thus being of great importance in the understanding and treatment of those fundamental functional recovery mechanisms triggered after stroke.

The present chapter will focus on standard, current and new potential treatments of acute phase, prevention and rehabilitation of IS.

INTRODUCTION

Ischemic stroke (IS) is the consequence of a critical reduction of cerebral blood flow in a localized brain region resulting from the sudden occlusion or progressive narrowing of a large or small cerebral artery [1]. The result is the definitive (IS) or temporary (transient ischemic attack or TIA) loss of function of the affected region in the brain with consequent appearing of neurological deficits such as facial drop, slurred speech, speech arrest, weakness of the arm and / or leg on the same side.

It is estimated that 530000 people experience a new IS each year in the USA and on average every 40 seconds someone in the same country has a stroke [2]. In terms of mortality stroke ranks number 4 among all cause of death after heart disease, cancer and chronic lower respiratory disease [3]. However it remains the first cause of adult neurological disability in developed countries [4]. About 80% of patients come back home but about half of them needs permanent or temporary help in the home setting [5]. In fact with improvement of treatment strategies, more patients survive so that the annual stroke rate decreased 34.3% from 1997 to 2007 and actually 18.8%. Unfortunately many of stroke patients remain with physical and cognitive sequelae with consequent increased personal and public costs [2,5]. In this regard, a recent estimation of the total direct (cost of physicians and other professionals, hospital services, prescribed medications, home health care) and indirect (lost of productivity/mortality) costs deriving from stroke resulted in about 41 billions of dollars with an overall cost for all cardiovascular diseases estimated to be about 286 billions of dollars [6].

Data from the Framingham Heart Study showed that stroke incidence is declining over time: in particular the age-adjusted incidence of first stroke per 1000 person-years has decreased from 7.6 for men and 6.2 for women in the period 1950-1977 to 6.2 for men and 5.1 for women in the period 1990-2004 [7].

However, a recent systematic review showed over the past four decades a 42% decrease in stroke incidence in high income countries and a greater than 100% increase in stroke incidence in low-to-middle income countries [8]. On the contrary, stroke severity did not vary across these periods [7]. The risk of first-ever stroke in blacks is almost twice than in whites and this racial disparity doesn't seem to change over time. Socioeconomic condition has been hypothesized to be implicated in the excess stroke incidence in blacks [9].

Among stroke risk factors, TIA confers an important short-term risk of stroke (10% within 90 days and 5% within 2 days) [10]; hypertension plays a crucial role in the risk of both ischemic stroke and intracranial hemorrhage [11]. Diabetes mellitus nearly triples while current cigarette smoking doubles this risk [12]. Atrial fibrillation, although often asymptomatic and undetected, is an important risk factor for stroke, increasing stroke risk

about 5-fold throughout all ages so that its relevance could be underestimated [13,14]. Patients with low concentrations of HDL cholesterol have been found to be at higher risk of stroke [15]. Further depressive symptoms have been increasingly recognized as a risk factor (4-fold higher) for stroke/TIA [16]. Clinical and experimental research are aimed at counteracting the global impact of IS in terms of economic and disability burden. Over the last decades, three different aspects of IS management have been deeply investigated: primary and secondary prevention, acute treatment and rehabilitation. The present chapter will focus on each one of the aforementioned phases of IS management, highlighting all current recommended procedures and potential new developments.

ACUTE ISCHEMIC STROKE TREATMENT

IS includes a region characterized by a decrease in blood flow below the threshold of energy failure which is called ischemic core and a surrounding area called ischemic penumbra (IP) (figure 1). The latter has been regarded as a region in which reduction in blood flow goes under the threshold for failure in electric function but not for membrane failure. The main concept deriving from this assumption is that the tissue in this region is still viable [1,17].

Cerebral blood flow studies based on magnetic resonance imaging (MRI) and positron emission tomography (PET) have shown that the IP represents a potentially reversibly damaged tissue, while its conversion to complete infarction is responsible for acute clinical deterioration in stroke patients [1,18]. For the same reason IP represents the main target of acute IS treatment whose purpose is therefore to reduce the amount of IP that can become irreversibly damaged thus enlarging the IC.

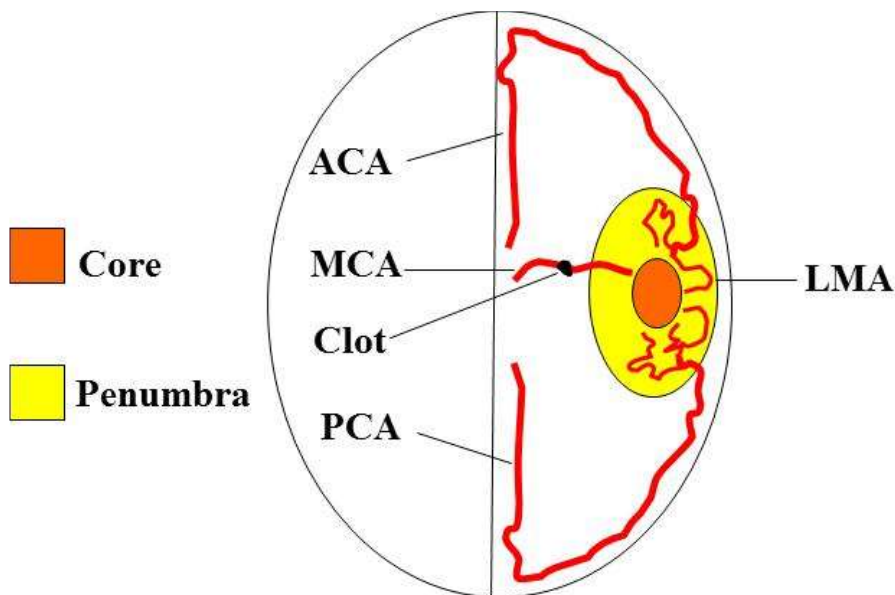


Figure 1. Schematic representation of blood supply to the penumbral tissue through leptomeningeal anastomoses (LMA). ACA: anterior cerebral artery; MCA: middle cerebral artery; PCA: posterior cerebral artery.

Up to now, the only drug authorized for the acute stroke treatment is a thrombolytic agent called recombinant tissue plasminogen activator (rtPA) administered intravenously (I.V.) within 3 hours of symptom onset. Endogenous tPA is a serine protease (found in endothelial cells) that catalyzes the conversion of plasminogen to plasmin. The latter is responsible for conversion of fibrin clot in fibrin degradation products and clot breakdown [19]. Compared to the first generation plasminogen activators (streptokinase and urokinase), rtPA has a higher affinity for fibrin and is activated by fibrin with consequent activation of plasminogen on the fibrin surface. Thus due to this fibrin specificity, rtPA is able to induce effective thrombolysis without enhancing systemic plasma proteolysis [20,21]. Therefore, the aim of thrombolytic therapy is to lyse an occluding thrombus or embolus, to restore function in potentially still-viable tissue and to reduce the volume of cerebral tissue irreversibly damaged.

The trigger for the Food and Drug Administration (FDA) approval of rtPA in acute stroke treatment in 1996 came from the results of the National Institutes of Neurological Disorders and Stroke (NINDS) Trial (1995) which demonstrated that i.v. administration of rtPA at a dosage of 0.9 mg per kilogram of body weight (10% bolus and resting dose in 1 hour) was able to increase the number of patients with good clinical outcome as compared with the untreated group [22]. In particular, treated patients were at least 30 percent more likely to have minimal or no disability at 3 months, and this effect was confirmed at 1 year follow-up [23].

However, contrary to its successful employment in acute myocardial infarction, one of the main complications of thrombolysis in stroke is intracerebral haemorrhage (ICH) which can offset any beneficial effects [24]. This complication, together with the difficulty to start treatment within three hours of symptom onset, led to a general criticism about this approach. Further, the NINDS trial was the first and only randomized-controlled trial assessing the usefulness of a thrombolytic agent in acute stroke treatment showing positive results. For all these uncertainties, approval of the European Agency for the evaluation of medical product (EMA) came only in 2002 under condition that i.v. thrombolysis was only used in observational studies and that a new randomized placebo controlled trial was started to evaluate safety and efficacy of i.v. rtPA in the 3-4,5 hours time window. In the following years the Safe Implementation of Treatments in Stroke Monitoring Study (SITS-MOST) registry [26] with 6,483 patients treated with rtPA within 3 hours of symptom onset, confirmed the NINDS results. Other large studies such as the Alteplase Thrombolysis for Acute Non Interventional Therapy in Ischemic Stroke (ATLANTIS) and the European Cooperative Acute Stroke Study (ECASS) further reported the beneficial effects of iv thrombolysis within 3 hours of symptom onset [27,28].

A the short therapeutic time-window is considered the principal responsible of the low (<5%) percentage of treated patients [29]. Many efforts have been made to enlarge the therapeutic time-window. In 2004 a metanalysis of the ATLANTIS, ECASS and NINDS trials [30] investigated the relationship between onset-to-treatment time (OTT) and both favorable 3-month outcome and occurrence of symptomatic ICH (sICH). With 2,775 patients randomly assigned to rtPA or placebo within 360 min of stroke onset, this analysis showed that odds of favorable 3-month outcome increased as OTT decreased with some benefits also beyond the 3-hour window; however the hazard ratio for death was similar for different intervals between 0-270 min while increased in the 271-360 min interval, indicating that, although the potential benefit of rtPA beyond 3 hours, this potential could come with some risks (figure 2).

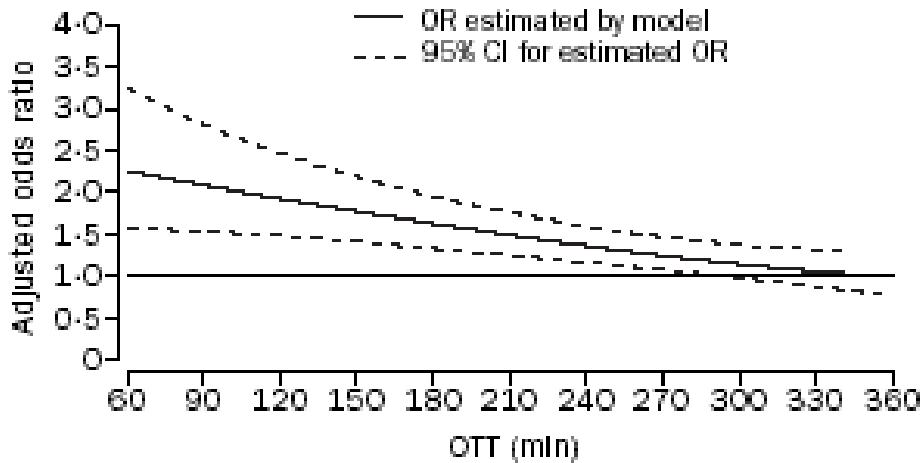


Figure 2. The relationship between onset-to-treatment time and odds ratio of favorable outcome.

Haemorrhagic complications were seen in 5.9% of rtPA patients and 1.1% of controls and was associated with rtPA treatment and age but not with OTT. In light of this analysis and as requested by the EMEA, in 2008 the ECASS III investigators published the results of a large randomized controlled trial aiming to evaluate safety and efficacy of rtPA administered between 3 and 4.5 hours after the onset of stroke [31]. 821 patients were enrolled and randomly assigned to rtPA group (418) and to the placebo group (403).

More patients had a favorable outcome with rtPA than with placebo (52.4% vs 45.2%; $p=0.04$) and the outcome was also better with rtPA than with placebo. The incidence of ICH (either any or symptomatic ones) was higher with rtPA than with placebo, while mortality did not differ between the two groups. In the same year, another large study called the Echoplanar Imaging Thrombolytic Evaluation Trial (EPITHET) [32] aimed to evaluate whether alteplase, administered between 3 and 6 hours after stroke onset, was able to promote reperfusion and to attenuate infarct growth in patients with demonstrated “still savable tissue” (IP) on MRI. 101 patients were randomized to Alteplase or placebo. The study showed that Alteplase was non-significantly associated with lower infarct growth (calculated on MRI) and with significant reperfusion in patients with demonstrated IP and that reperfusion was associated with better clinical outcome.

Therefore an updated metanalysis, adding data from recent EPITHET and ECASS III trials to those of ATLANTIS, ECASS and NINDS, re-examined the effects of OTT on benefits and risks of Alteplase administration [33]. With 3,670 patients, the analysis confirmed that patients benefit from i.v. rtPA up to 4.5 hours and that sooner the treatment was started, the greater the benefit, especially within 90 minutes. Again, the study showed that beyond the 4.5 hours, the risk could outweigh benefit. Despite all these studies and the supposed improvement in the rate of patients undergoing i.v. thrombolysis as a consequence of widened therapeutic window, a recent study [34] reported that of 1,838 patients only 3.4% arrived in the expanded time interval compared with the 22% arrived in the standard time window. Moreover, only 0.5% from the expanded time frame met eligibility criteria for thrombolysis compared to 5.9% in the standard time frame. Hence, only few patients seem to benefit from the results of ECASS III trial and problems other than the short time therapeutic

window are likely involved in the low rtPA administration rate such as: poor stroke awareness and impaired transport to facilities with ability to administer Alteplase and poor familiarity of physicians with acute stroke treatment guidelines. A way to improve the rate of rtPA administration has been explored in a study which took into account that many contraindications included in the license of Alteplase are not based on scientific evidence, while they restrict significantly the portion of patients with acute IS eligible for treatment with Alteplase [35]. The authors tested whether off-label thrombolysis (patients treated out of license indications for Alteplase) was associated with poorer outcome or increased rate of sICH compared with on-label use (patients from the large randomized controlled trials). They found that more than 50% of treated patients had contraindications to thrombolysis and that off-label treatment was neither associated with poorer outcome (except for age > 80 years), nor with increased rate of sICH when compared with on-label patients. Some innovative aspects have to be taken into consideration from this study in order to find out strategies to overcome the low rate of rtPA administration: 1. more than 10% of patients with minor stroke (a contraindication for the license of Alteplase) were treated thus improving definitely the rate of i.v. thrombolysis treated patients; 2. in order to shorten as much as possible the OTT, there was no reason to wait for laboratory results unless an history or clear clinical sign suggestive of those laboratory abnormalities considered as a contraindication for treatment [35].

From all these data come the needs to start i.v. thrombolysis as soon as possible since “time lost is brain lost”. In this view, in order to enhance and expedite acute stroke care, an important support has been telemedicine [36]. Telemedicine represents a technology which allows the remote interaction and exchange of audio-visual information between physicians at different places [37]. Because of a shortage of stroke specialists, this technology carries the potential of allowing many outlying or “spoke” hospitals to initiate thrombolytic treatment before transferring patients to a regional stroke center hub thus avoiding harmful delay [36]. One of the largest study testing safety and efficacy of telemedicine in stroke, also called telestroke, comes from Germany and in particular from the rural area of Bavaria with its telestroke network composed of 2 stroke centers and 12 local hospitals [38]. The authors designed the study considering that: long distances in rural area may prevent timely admission to the hub; from the German Registers Study Group thrombolysis in local hospitals resulted in increased complication rates [39]; international guidelines suggest that rtPA should only be given if stroke diagnosis is made by a physician with expertise in stroke [40]. Based on this assumption, the authors found a marked increase in the rate of treated patients in local hospitals and a rate of sICH and in-hospital mortality lower than that reported in inexperienced regional hospitals [38].

In the past few years many efforts have been made to evaluate the economics of treating acute IS. This is due to the relevance of stroke as a cause of long-term disability. In the years next to the Alteplase approval it was observed that acute costs of hospitalization and thrombolysis were offset by the greater likelihood of a favorable recovery and thereby a decrease in rehabilitation costs with a net cost savings to the healthcare system [41]. Despite the initial enthusiasm, a discouragement came with the recognition that only 2% of stroke patients were actually treated with rtPA. However, in the last few years, a net increase in the proportion of patients receiving Alteplase has been observed probably due also to the establishment of stroke networks (hub and spoke) and of telestroke [42]. Finally, economics of acute IS treatment proposed that the higher the rtPA administration rate the more remarkable the cost saving. It goes parallel to this concept the relevance of starting treatment

as soon as possible since, as already described, the earlier the treatment the higher the likelihood of good clinical outcome and reduced rehabilitation costs [33,41].

One of the biggest shortcoming of i.v. thrombolysis is the poor outcome obtained in patients with IS secondary to large intracranial arteries occlusion [43,44]. In particular a recent study [45] evaluated the association between the clot location in the intracranial arteries and the likelihood of good clinical outcome after i.v. thrombolysis and found that outcome improved and mortality decreased when moving from proximal (internal carotid artery) to more distal vessel (distal segments of middle cerebral artery) position and that a cut-point between the proximal and distal part of the middle cerebral artery was associated with the largest increase in odds for favorable outcome. The poor outcome, together with the need of extending the therapeutic window, has been the trigger for research in other recanalization strategies. Endovascular therapy represents a current challenge in the treatment of acute IS in the last few years despite devices, specifically designed for this purpose, were first developed in the 1990s and intra-arterial thrombolysis has been used for nearly three decades [46]. The latter has been evaluated in only one randomized trial called Prolyse in Acute Cerebral Thromboembolism II (PROACT II) testing safety and efficacy of this therapy⁴⁷. Patients were randomized to intra-arterial prourokinase with i.v. heparin versus i.v. heparin alone. This trial showed a 66% rate of recanalization and a 40% good neurological outcome. Nowadays these results remain the yardstick by which newer interventional strategies are judged. However, in a meta-analysis of randomized controlled trials evaluating intra-arterial fibrinolysis for acute IS, PROACT II was the only to show a statistically significant clinical benefit but resulted in a net discrepancy between recanalization of occluded cerebral arteries and clinical outcomes since the first had a rate 3 times higher than that of favorable outcome [48]. Moreover prourokinase is no longer available and intra-arterial fibrinolysis is an off-label application of any available fibrinolytic agent since it requires further investigation for on-label application [46,47]. Driven by the knowledge that recanalization (therapeutic or spontaneous) and reperfusion in acute IS are associated with improved outcome and reduced mortality [49], alternative endovascular methods have been investigated such as mechanical thrombectomy. All the devices designed for this strategy offer potential advantages such as more rapid recanalization, ability in recanalization of large-artery occlusions, reduced use of pharmacological thrombolysis with lower risk of ICH and extended therapeutic time-window compared with i.v. thrombolysis [50]. Five kind of neurothrombectomy devices exist: clot retrievers, aspiration or suction devices, snare-like devices, ultrasonography technologies, lasers. Potential harms include direct trauma to the vasculature (vasospasm, dissection, perforation, rupture) and thrombi fragmentation into previously unaffected vessels [51]. Another risk of the procedure itself is represented by the frequent need for intubation and heavy sedation which have been shown to be associated with worse outcome [52]. In particular, a recent retrospective analysis correlated general anesthesia with lower systolic blood pressure values and identified local anesthesia and systolic blood pressure greater than 140 mmHg as independent predictors of good clinical outcome [53]. Currently, only two devices are cleared by the FDA:

1. the Mechanical Embolus Removal in Cerebral Ischemia (MERCII) which is a clot retriever and was the first to be cleared and evaluated;
2. the Penumbra System (PS) which is an aspiration device [54].

Both them have the indication to recanalize the artery within 8 hours of symptom onset. By crossing the site of occlusion, the MERCI retriever pulls the embolus into an extracranial guide catheter under active suction [46] (Figure 3). This device has been tested in 3 prospective, single-arm, nonrandomized studies enrolling patients ineligible for or non-responding to i.v. thrombolysis within 8 hours of stroke onset. Primary outcome included partial or complete recanalization and safety while secondary outcomes included clinical outcome at 30 and 90 days. The studies showed a rate of successful recanalization of 46% that was significantly higher than that expected by using the control arm of the PROACT II (18%) and a rate of good neurological outcomes of 28%; moreover recanalization was associated with good outcome whereas increased age and stroke severity with poor outcome and death [55]. In particular a pooled analysis of the MERCI and multi-MERCI trials highlighted a marked increase in the rate of good clinical outcome in patients with arterial recanalization as compared to the non-recanalized group, identifying internal carotid artery recanalization as a significant predictor of good-90-day outcome and failure in recanalization as a significant predictor of mortality at 90 days [56-58]. In all these studies rates of successful recanalization ranged between 43% and 78%. The PS acts on the proximal face of the clot without crossing it and is used in combination with an aspiration device to debulk and extract the clot (Figure 4). It has been tested in the Penumbra Pivotal Stroke Trial [59] which was a prospective, single-arm, multicenter study that showed a further increase in the rate of successful recanalization (81.6%) compared with the previous trials [59].



Figure 3. Merci retriever.

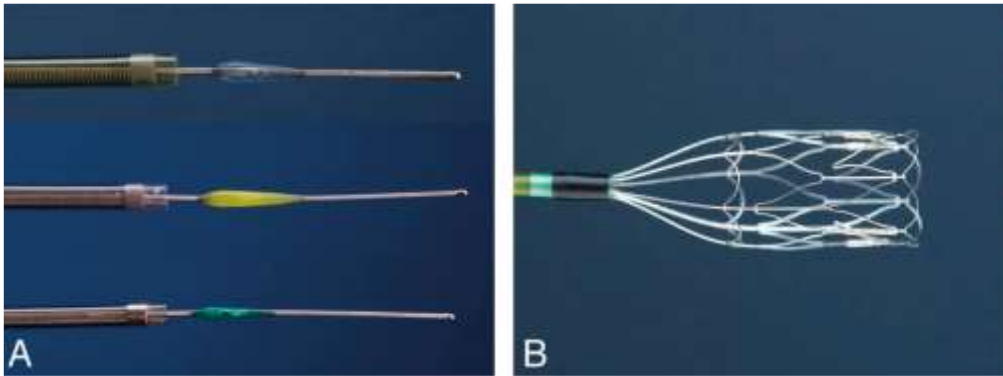


Figure 4. Penumbra System.

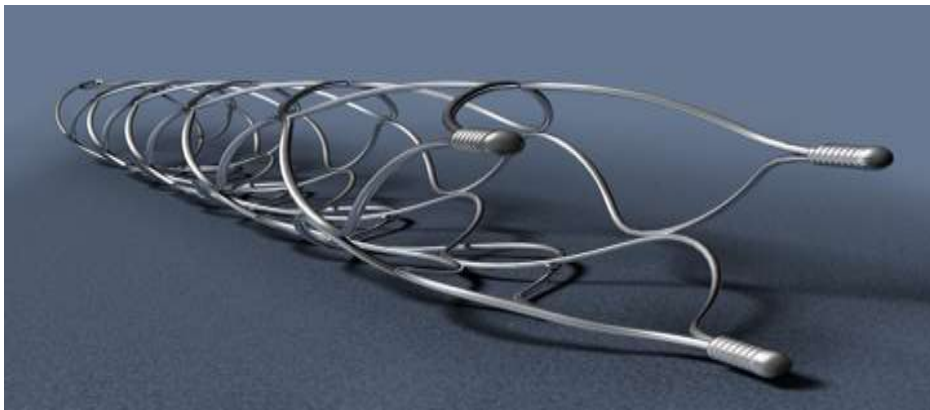


Figure 5. Solitaire device.

Yet, the rate of good clinical outcome was only 25%. Further increase in successful recanalization rate has been observed in a recent study [60] evaluating safety and efficacy of a new device called Solitaire. This is a self-expanding stent that can be fully deployed and then retrieved thus allowing mechanical embolectomy (Figure 5). The authors described impressive results with a rate of successful recanalization of 88% and a rate of favorable outcome of 70% for MCA occlusion, 44% for ICA occlusion and 43% for basilar artery occlusion with an overall rate of 54%. Finally, the overall mortality rate was 12%. Due to its technical characteristics, some risks linked to the use of Solitaire could be the dissection of distal branches and distal dislocation of the clot during the thrombus retrieval.

Although promising results are coming from research in endovascular therapy, mortality rate in treated patients remains around 35% [46]. As already described, some predictors of unsuccessful recanalization have to be taken into account such as poor collateral cerebral circulation, high pre-treatment stroke severity, age > 70 years and elevated clot burden [61,62]. Thus, uncertainty remains about which kind of patient population is the most likely to respond to endovascular therapy. Therefore, a careful patient selection probably based on advanced imaging techniques able to quantify the ischemic core and the ischemic penumbra as well as to evaluate collateral circulation, should be considered for future trials. In our opinion, since successful recanalization seems to correlate with favorable neurological

outcome, the combination of all endovascular strategies currently available should be taken into consideration, at least until results of ongoing and future trials will clarify the superiority of one strategy on another.

FUTURE DIRECTIONS AND CONCLUSION

IS represents an increasing economic burden. Prevention of disability seems to be the way of counteracting healthcare costs through a reduction in disability and associated long-term care costs [63]. Endovascular therapy seems to be a further step, after i.v. thrombolysis, in that direction since its potentials should be to widen temporal therapeutic window beyond 4,5 hours and to achieve a more rapid recanalization, thus increasing the number of treated patients. In this regard training of interventional specialists represents a big challenge since success is directly related to the time of recanalization [64]. However, some authors have recently underlined the lack of relationship between the grade of recanalization and the functional outcome obtained with endovascular therapy, while others have supported the importance of a good pre-treatment collateral flow as an independent predictor of good clinical outcome [61,65]. These authors reported also a higher frequency of infarct growth and of symptomatic haemorrhagic transformation in patients with poor collaterals and achieved recanalization, thus supporting the prognostic value of collateral flow [61]. It has been shown that collateral flow can sustain brain tissue for hours after arterial occlusion and maintenance or potentiation of collateral flow could be regarded as a potential therapeutic target [66,67]. In our opinion some technologies/strategies should be considered worthy of attention in the modulation of collateral flow in the acute phase of ischemic stroke. One of these is repetitive transcranial magnetic stimulation (rTMS) which demonstrated to improve cerebrovascular reactivity (CVR), a marker of perfusion autoregulation ability, in healthy volunteers as demonstrated by our group [68]. Although currently insufficient data exist on the safety and efficacy of statins in acute IS in humans [69], their helpful effect in this specific scenario could be favored by their fibrinolytic action, endogenous nitric oxide synthase (eNOS) upregulation (with increased NO production and consequent potentiation of CVR), increased cerebral blood flow and platelet deactivation [67]. As well as statins, some phosphodiesterase inhibitors such as sildenafil and cilostazol have shown the ability to improve CVR [70,71], to promote angiogenesis, endothelial function and antiplatelet effects thus presenting potentials in the modulation of collateral flow in the early phase of IS [67]. We argue and emphasize our point of view that the need for early recanalization in acute IS has to be considered as a valid reason for combination of all current available strategies with demonstrated success in arterial recanalization, as it has already been elaborated in the design of the Interventional Management of Stroke Phase III trial [72].

STROKE PREVENTION: MANAGING MODIFIABLE RISK FACTORS

Prevention plays a crucial role in counteracting morbidity and mortality related to IS. It has been estimated that 50% of stroke are preventable through control of modifiable risk factors and life-style changes. Stroke Prevention has been recently set as one of the priorities

by an international community of leaders involved in this field) [73], and the American Heart Association (AHA) and American Stroke Association (ASA) have recently published updated guidelines for recurrent stroke prevention [74]. Over the last four decades several certainties have been achieved. Primary prevention strategies that work in primary prevention of IS are: treating hypertension (HTN), and using statins and angiotensin-converting enzyme inhibitors (ACEIs). Attention to lifestyle factors is routinely warranted in both primary and secondary IS prevention: aerobic exercise to counteract inactivity, weight loss in obesity, glucose control in diabetics, smoking cessation, and diet. Patients who have had an IS or transient ischemic attack (TIA) are at high risk for recurrent stroke.

Antihypertensive treatment is recommended for both prevention of recurrent stroke and other vascular events. Cholesterol lowering with statins, and antiplatelets have been shown to reduce the risk of recurrent stroke and other vascular events; ACEIs or Angiotensin II receptor blockers (ARBs) are indicated in stroke prevention because they promote vascular health; effective secondary-prevention strategies for selected patients include carotid revascularization for high-grade carotid stenosis and vitamin K antagonist (i.e., warfarin) treatment for atrial fibrillation. Among potentially modifiable risk factors, consensus does not exist on the role of treating, among others, hyperhomocysteinemia, coagulation disorders, patent foramen ovale, metabolic syndrome, lipoprotein(a), and markers of inflammation to prevent stroke. The results of recent clinical trials investigating new anticoagulants (factor Xa inhibitors and direct thrombin inhibitors) clearly indicate alternative strategies in stroke prevention for patients with atrial fibrillation. Interesting data on the phosphodiesterase inhibitors cilostazol are emerging and need confirmation in randomized controlled studies (RCTs). This review describes the current landscape and developments in stroke prevention with special reference to medical treatment in secondary IS prevention.

TREATMENT OF HYPERTENSION

Arterial hypertension (HTN) is the single most important modifiable risk factor for stroke. HTN contributes to 60% of all strokes (through the following mechanisms: atheroma in carotids, vertebral arteries and aortic arch; friability of small cerebral arteries; left ventricular dysfunction and atrial fibrillation). There is a close, continuous and approximately linear relationship between blood pressure (BP) levels and primary incidence of stroke in both hypertensive and normotensive populations. A 5-year reduction of 5-6 mmHg diastolic blood pressure (DBP) (with mainly diuretics and beta-blockers) has been associated with a 42% relative risk reduction (RRR) of first stroke [75]. Randomized trials on primary stroke prevention in middle-aged and elderly populations confirmed that treating HTN reduces the incidence of stroke. Two placebo-controlled trials (the first using chlorthalidone and atenolol, the second using nitrendipine, with the possible addition of enalapril and hydrochlorothiazide) have demonstrated 36% [76]. and 42% [77] RRR for first ischemic stroke in treating systolic blood pressure (SBP) as compared to placebo. More recently, the LIFE study [78] compared losartan-based regimen versus atenolol-based regimen in 9,193 hypertensive patients with a mean follow-up of 4.8 years. While comparable reductions in BP were observed, losartan treatment was associated with a significant 25% risk reduction versus atenolol of fatal and non fatal stroke. Losartan was better tolerated and seemed to confer benefits beyond reduction

in BP. A recent analysis of RCTs compared first-line calcium channel blockers (CCBs) with other antihypertensive classes, in order to determine whether CCBs reduced the incidence of major adverse cardiovascular events compared to the other antihypertensive classes [79]. Eighteen RCTs, with at least 100 randomized hypertensive participants and with a follow-up of at least two years, with a total of 141,807 participants, were included. Although some results were not enough robust to change practice, CCBs reduced stroke as compared to beta-blockers, ACEIs, and ARBs. However, diuretics were the preferred first-line treatment over CCBs to optimize reduction of cardiovascular events and congestive heart failure.

Few trials have focused on antihypertensive therapy for prevention of recurrent stroke. Two studies using ACEIs have demonstrated that, for patients with hypertension, effective control of BP reduces the risk of recurrent stroke. The Perindopril Protection against Recurrent Stroke Study (PROGRESS) [80] was started in 1996 to answer this question. In this study, about 6,000 hypertensive patients with a prior TIA or stroke in the last 5 years were randomized to receive aggressive HTN treatment (perindopril 4 mg±indapamide 2.5 mg) versus usual care (1 drug) or placebo (1 or 2 placebo). Patients were followed up for 4 years, and the primary outcome was total strokes. Aggressive treatment was associated with a 43% RRR in stroke risk versus usual care (mean BP reduction was 12/5 and 5/3 mmHg). The HOPE trial [81] was a RCT, with 267 participating hospitals in 19 countries. There were 9,297 patients with vascular disease or diabetes plus an additional risk factor, followed-up for 4.5 years (11% of them had prior TIA or IS). Patients were randomized to receive ramipril 10 mg versus placebo. The rate of stroke and TIA were assessed. Reduction in BP was modest (3.8 SBP and 2.8 DBP). The RRR for any stroke was 32%, and the RRR of fatal stroke was 61% (17 versus 44 events) favoring ramipril. Benefits were consistent across patients' subgroups.

More recently, other trials have tested safety and efficacy of ARBs, when used alone or in combination with an ACEI, in preventing stroke recurrence in high-risk populations. In the PReventiOn regimen For Effectively avoiding Second Strokes (PROFESS) trial [82], 20,332 ischemic stroke patients were randomized to telmisartan 80 mg or placebo. After a mean treatment of 2 years, telmisartan showed a non significant lower rate of recurrent stroke versus placebo [880 versus 934; hazard ratio (HR) 0.95; 95% confidence interval (CI) 0.86-1.04]. However, in a post-hoc analysis, a significantly reduced number of strokes was observed in the telmisartan group compared to placebo (533 versus 608; HR 0.88; 95% CI 0.78-0.99; $p=0.042$) [83]. The ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial (ONTARGET) and The Telmisartan Randomized AssessmeNt Study in aCE-iNtolerant subjects with cardiovascular Disease (TRANSCEND), compared telmisartan 80 mg versus ramipril 10 mg, and telmisartan 80 mg versus placebo (in patients intolerant to ACEIs), respectively. In the stroke subgroup, telmisartan 80 mg showed a trend toward reducing recurrent stroke versus ramipril 10 mg (HR 0.91; 95% CI 0.79-1.05). In a combined analysis of PROFESS and TRANSCEND, the incidence of the composite of stroke, myocardial infarction, or vascular death was 12.8% for telmisartan versus 13.8% for placebo (HR 0.91; 95% CI 0.85-0.98; $p=0.013$) [84].

The MOSES study showed for the first time superiority of an ARB (eprosartan) compared with a calcium channel antagonist (nitrendipine) in antihypertensive treatment for secondary stroke prevention [85]. In this study, 1,405 high-risk hypertensive stroke patients

were randomized. BP was reduced to a comparable extent without any significant differences between the 2 groups (from 150.7/84 mm Hg and 152.0/87.2 mm Hg with eprosartan and nitrendipine therapy to 137.5/80.8 mm Hg and 136.0/80.2 mm Hg, respectively). For the same level of BP control, eprosartan was significantly more effective than nitrendipine in reducing cerebrovascular morbidity and mortality (102 strokes in the eprosartan and 134 in the nitrendipine group; $p=0.03$).

Based on all these studies, the renin-angiotensin system (RAS) blockers (ACEIs and ARBs) are guideline-recognized, highly effective antihypertensive agents which offer benefits that extend beyond BP reduction alone. Experimental and clinical data suggest that reducing the activity of the RAS may have cerebroprotective effects. The Angiotensin II has hemodynamic properties (potent peripheral vasoconstrictor, stimulates aldosterone), action on endothelium (mediates endothelium dysfunction, vascular smooth cells hypertrophy), stimulates oxidative stress, including oxidation of low-density lipoprotein cholesterol (LDL-C), and inflammation (associated with expression of cellular adhesion molecules, chemotactic and proinflammatory cytokines) thus contributing to endothelial damage and arterial wall injury [86]. In a recent meta-analysis, after controlling for effects on BP control, ARBs appeared to be more effective than either ACEIs or β blockers in stroke prevention; however, CCBs were superior to RAS blockers in stroke prevention [87].

In conclusion, epidemiological studies and clinical trials confirmed the hypothesis of managing HTN to counteract the risk of stroke. The recommendation [74] to prevent recurrent stroke is to treat HTN aggressively (Class I, Level of Evidence A); although an absolute target of BP level has not been clearly defined, benefit has been associated with an average reduction of 10/5 mmHg, and normal BP levels have been defined as <120/80 mm Hg (Class IIa, Level of Evidence B). These recommendations extend to all patients with prior IS or TIA, irrespective of history or HTN, if BP reduction is considered appropriate (Class IIa, Level of Evidence B). Studies aimed at comparing different antihypertensive drugs are insufficient and have not reached clear results, but what is clear is that the effects of antihypertensive go beyond the simple control of BP. Diuretics alone or in combination with an ACEI are indicated (Class I, Level of Evidence A). The choice of a specific drug should be individualized based on drug and patient characteristics (extracranial occlusive disease, renal impairment, cardiac disease, and diabetes) (Class IIa, Level of Evidence B).

ATRIAL FIBRILLATION

Patients with non-valvular atrial fibrillation (AF) are at increased risk of stroke [13] [14]. The efficacy of warfarin over placebo has been consistently demonstrated across studies yielding an overall RRR of 68% (95% CI, 50% to 79%) and an absolute risk reduction (ARR) in annual stroke rate from 4.5% for controls to 1.4% in patients with adjusted-dose warfarin: 31 ischemic strokes will be prevented each year for 1000 patients treated [74]. Warfarin use has been also associated with a modest 1.3% annual rate of major bleeding as compared with 1% for patients on placebo or aspirin.

As compared to warfarin, a weaker efficacy of aspirin has been demonstrated in a pooled analysis of three clinical trials [88]. Analysis of an additional arm of the Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events (ACTIVE A) in patients

“unsuitable for vitamin K antagonists therapy” [89] showed superiority of combination therapy with aspirin and clopidogrel over aspirin alone in reducing the rate of stroke (2.4% vs 3.3% per year; $p > 0.001$). However, based on the observation that the rate of major vascular events combined with major hemorrhages did not significantly differed between the two groups, aspirin remains the treatment of choice in patients with AF and a clear contraindication to vitamin K antagonists. The European Atrial Fibrillation Trial (EAFT) [90] demonstrated superiority of anticoagulation over aspirin in preventing stroke recurrence in patients with history of IS or TIA: anticoagulation was significantly more effective than aspirin (HR 0.60; 95% CI 0.41-0.87). The incidence of major bleeding events was higher in the anticoagulation group, but low in both groups (2.8% and 0.9% per year); in absolute terms: 90 vascular events (mainly strokes) could be prevented if 1000 patients were treated with anticoagulation for one year. Aspirin demonstrated to be a valid, although less effective, alternative when anticoagulation was contraindicated, preventing 40 vascular events each year for every 1000 treated patients. More recently, systematic review of primary prevention studies in patients with non-valvular AF, demonstrated that adjusted-dose warfarin and related oral anticoagulants reduced stroke, disabling stroke and other major vascular events by about one third when compared with antiplatelet therapy [91]. For patients with AF who suffer an IS or TIA despite therapeutic anticoagulation, increasing the intensity of anticoagulation or adding an antiplatelet does not provide additional protection in preventing stroke while increases the risk of bleeding [88] [91].

Several new antithrombotic agents have been developed for stroke prevention in patients with non-valvular AF. New drugs for oral anticoagulation that do not exhibit the limitations of vitamin K antagonists include direct factor Xa inhibitors and direct thrombin inhibitors. In the Stroke Prevention Using Oral Thrombin Inhibitor in Atrial Fibrillation (SPORTIF) III (open-label, $n=3,407$) [92] and V (double-blind, $n=3,922$) [93], safety and efficacy of the oral direct thrombin inhibitor ximelagatran (fixed dose, 36 mg twice daily) were compared to warfarin (adjusted dose, target international normalized ratio [INR] 2.0-3.0) in patients with nonvalvular AF and at least 1 risk factor for stroke. Pooled analysis showed that the efficacy of ximelagatran was comparable (noninferior) with extremely well-controlled warfarin therapy in preventing stroke and systemic embolic events; the primary event rates were 1.65% per year and 1.62% per year in the warfarin and ximelagatran groups, respectively ($p=0.941$). In patients with a history of stroke or TIA (about 20% of the SPORTIF population), the event rates were 3.27% per year and 2.83% per year in the warfarin and ximelagatran groups, respectively ($p=0.625$). Intracranial hemorrhage occurred at a rate of 0.20% per year with warfarin and 0.11% per year with ximelagatran. Combined rates of minor and major bleeding were significantly lower with ximelagatran than with warfarin (32% per year vs 39% per year; $p < 0.0001$). The authors concluded that ximelagatran administered without coagulation monitoring or dose adjustment was as effective as well-controlled, adjusted-dose warfarin for prevention of stroke and systemic embolic events and was associated with significantly less total bleedings [93].

More recent trials have compared warfarin to dabigatran (RE-LY) [94], rivoraxaban (ROCKET-AF) [95], and apixaban (ARISTOTLE) [96], while in the AVERROES [97], safety and efficacy of apixaban (at a dose of 5 mg twice daily) was investigated as alternative treatment to aspirin (81 to 324 mg per day) in patients who were not suitable candidates for or were unwilling to receive vitamin K antagonist therapy. Taken together, these trials have demonstrated similar efficacy in patients with AF of these novel anticoagulants in both

primary and secondary stroke prevention, with significantly lower incidences of intracranial bleeding, compared with warfarin. Of note, the AVERROES was stopped prematurely because of a clear benefit in favor of apixaban was observed: apixaban reduced the risk of stroke or systemic embolism without significantly increasing the risk of major bleeding or intracranial hemorrhage. Based on the results of the RE-LY study [94], and with a modest absolute reduction of stroke or systemic embolism (1.7% versus 1.1%, $p < 0.001$), the most successful alternative anticoagulant is dabigatran, given at a dose of 150 mg twice daily.

A systematic review and meta-analysis of RCT has been recently performed to compare the efficacy and safety of new oral anticoagulants to those of warfarin in patients with AF [98]. The authors identified 3 studies, including 44,563 patients. Patients randomized to new oral anticoagulants had a decreased risk for all-cause stroke and systemic embolism (relative risk [RR] 0.78, 95% CI 0.67 to 0.92), ischemic and unidentified stroke (RR 0.87, 95% CI 0.77 to 0.99), hemorrhagic stroke (RR 0.45, 95% CI 0.31 to 0.68), all-cause mortality (RR 0.88, 95% CI 0.82 to 0.95), and vascular mortality (RR 0.87, 95% CI 0.77 to 0.98). Randomization to a new oral anticoagulant was associated with a lower risk for intracranial bleeding (RR 0.49, 95% CI 0.36 to 0.66).

Based on these findings, new oral anticoagulants seem to be superior to warfarin in preventing stroke and systemic embolism in patients with AF. Further, they appear to have a favorable safety profile, making them promising alternatives to warfarin. However, a recent meta-analysis of RCTs has assessed safety and efficacy outcomes in patients with AF treated with warfarin for stroke prevention compared with an alternative thromboprophylaxis strategy [99]. Eight high-quality RCTs published in the last 10 years were selected, with a total of 32,053 patients included. The pooled analysis yielded 55,789 patient-years of follow-up. Overall, the time spent in the therapeutic range was 55% to 68%. The annual incidence of stroke or systemic embolism in patients with AF taking warfarin was estimated to be 1.66% (95% CI, 1.41%-1.91%).

Major bleeding rates varied from 1.40% to 3.40% per year across the studies. The risk of stroke per year was significantly higher in elderly patients (2.27%), female patients (2.12%), patients with a history of stroke (2.64%), and patients reporting no previous exposure to vitamin K antagonists (1.96%). The authors concluded that warfarin used as a stroke prevention agent in patients with AF was associated with a significantly lower rate of recurrent stroke or systemic embolism estimated compared to other antithrombotic treatments.

Conclusions: patients with IS or TIA with non-valvular AF should receive anticoagulation with a vitamin K antagonist (target INR 2.5, range 2-3) (Class I, Level of Evidence A); for patients unable to take oral anticoagulants, aspirin alone is recommended (Class I, Level of Evidence A); combination therapy with aspirin plus clopidogrel has the same contraindication of warfarin due to similar rate of bleeding complications (Class III, Level of Evidence B) [74].

TREATING HYPERLIPIDEMIA

Cholesterol levels represent an important and modifiable risk factor for coronary artery disease (CAD). However, the epidemiological association between cholesterol and stroke is

controversial: direct and moderately strong, direct but fairly weak, J shaped unclear and absent. Observational studies may be limited because cholesterol may have different effects on different stroke types; further, the incidence of stroke is lower and occurs later as compared to CAD. An association between serum cholesterol levels and both incident and recurrent stroke rate has not been clearly demonstrated. The Prospective Studies Collaboration [100] evaluated this association in pooled data of 45 prospective observational cohorts, with a total of 450,000 individuals, a mean follow-up of 16 years and a total of 13,397 strokes recorded. The analysis detected a 'flat effect' of increasing cholesterol levels on stroke risk. Despite only weak or no association of cholesterol levels with stroke, treatment with statins has consistently shown positive effects. The Scandinavian Simvastatin Survival Study (4S) [101] was a placebo-controlled trial which detected a 30% RRR in incidence of any stroke in patients treated with simvastatin as compared to placebo. In the Heart Protection Study (HPS) [102] 20,536 high-risk patients (CAD, occlusive arterial disease, diabetes mellitus) were enrolled in 69 UK hospitals: 13,379 (65%) with CAD, 3,280 (16%) with stroke+CAD, and 1,822 (9%) with stroke only. Patients were randomized to receive 40 mg simvastatin or placebo, and they had a follow-up of 5.5 years. Statin treatment was associated with a 27% RRR for all strokes, and a 25% RRR for ischemic stroke. However, no clear RRR in stroke recurrence was observed in patients with a prior stroke and no known CAD. A meta-analysis that included 16 statin trials with 34,000 patients and 860 strokes evidenced an overall 25% RRR for stroke (14% to 35%), with a non-significant 15% RRR (-28% to 43%) for primary, and a significant 35% RRR (18% to 49%) for secondary stroke prevention [103]. The Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) [104] evaluated secondary stroke prevention. The study demonstrated a 16% risk reduction of recurrent fatal or non fatal stroke in patients randomized to 80 mg atorvastatin versus placebo.

In secondary prevention of stroke, the use of statins has shown a positive effect in both decreasing progression and/or inducing regression of carotid artery plaque and stroke recurrence. By analyzing data from all available studies on statin treatment and stroke risk, Amarencu et al. [105] demonstrated a linear relationship between low-density lipoprotein-cholesterol (LDL-C) values and RRR of stroke. Each 10% reduction in LDL-C was estimated to reduce the risk of all strokes by 15.6% (95% CI, 6.7 to 23.6). This analysis also demonstrated a strong correlation between progression of carotid intima-media thickness (IMT) and LDL-C reduction.

The question is: why should statins prevent ischemic stroke? The possible explanations are two: lipid effects (LDL-C lowering), and ii) non-lipid effects. Among these the following have been demonstrated: i) stabilization of atherosclerotic plaque; ii) improvement of the endothelial function; iii) decrease in the inflammation; iv) decrease of platelet aggregation; v) a direct lowering effect of blood pressure and vi) a decrease in cardiac emboli, and vii) miscellaneous (reduced left ventricular hypertrophy, HTN, effects on endothelial progenitor cells). Experimental data suggests that LDL-C may damage the vascular endothelium by oxidation of lipids, glycation and oxidation of proteins. Its accumulation under the endothelium leads to activation of inflammation which triggers movements of macrophages and T cells into the intima, plaque development and progression, as well as development of a fibrous cap over the lipid core, which ultimately leads to plaque rupture and thrombus formation. Direct evidence of the molecular mechanisms modulating the plaque composition and its instability, and of a direct effect of statins on the inflammatory processes that are supposed to be involved in the plaque rupture have been demonstrated [106].

The association of total and high-density lipoprotein cholesterol (HDL-C) with stroke risk is unclear. This association has been recently investigated in among 58,000 Finnish people aged 25 to 74 years. During a mean follow-up period of 20.1 years, 3,914 participants developed incident stroke (3,085 were ischemic). Low levels of HDL-C and high total/HDL cholesterol ratio were associated with increased risks of ischemic stroke in both sexes. These associations attenuated after adjustment for body mass index, blood pressure, and history of diabetes [107]. Two recent meta-analyses have shown that ezetimibe co-administration with a statin provides significant additional lipid-lowering effect, allowing more patients to achieve low density lipoprotein cholesterol (LDL-C) target values [108] [109]. Although reduction of LDL-C remains the primary goal for lipid-lowering interventions, other targets (e.g., HDL-C and triglycerides) may also be important. However, there is no definitive evidence showing that raising HDL-C levels in patients on statins will result in a significant reduction in vascular events [110] [111]. Similarly, it is not clear if triglycerides levels are independent predictors of vascular risk [112].

Conclusions: statins are recommended to prevent the first stroke in high-risk patients to lower LDL-C level <100 mg/dL; an LDL-C <70 mg/dL is recommended for highest risk (high risk: any of LDL >4.1, age >45M/55F, positive family history, smoking, HTN, left ventricular hypertrophy; highest risk: DM or established atherosclerosis) (Class I, Level of Evidence A). New recommendation for secondary stroke prevention: on the basis of the SPARCL trial [74], statin treatment with intensive lipid-lowering effects is recommended to reduce the risk of stroke and cardiovascular events for patients with IS or TIA, evidence of atherosclerosis, an LDL-C level $\geq$ 100 mg/dL, and without known coronary artery disease (Class I, Level of Evidence B). In these patients, a target reduction of at least 50% in LDL-C or a target LDL-C level <70 mg/dL is recommended (Class IIa, Level of Evidence B). Patients with IS or TIA with low HDL-C may be considered for treatment with niacin or gemfibrozil (Class IIb, level B).

DIABETES

Epidemiological studies show that diabetes is a risk factor for first ischemic stroke, while data on stroke recurrence are more sparse [12] [74]. Further, the role of tight glycemic control in reducing the risk of stroke is still uncertain [113]. Patients with diabetes have higher mortality, more severe disability, and slower recovery after a stroke, as well as higher rates of stroke recurrence at 1 month (4.9% vs 2.6%) and at 2.6 years (15.2% vs 11.4%) compared to non-diabetic stroke patients [114] [115]. In addition, the ten-year risk of ipsilateral ischemic stroke after carotid endarterectomy is higher in the presence of diabetes (HR 2.24; 95% CI 1.35-3.74; $p=0.002$) [116]. The United Kingdom Prospective Diabetes Study (UKPDS) was a landmark study in the treatment of type 2 diabetes from the time of diagnosis [117]. This study has demonstrated that intensive treatment of type-2 diabetes versus standard treatment determined a 12% RRR the event rate (including stroke).

The Heart Outcomes Prevention Evaluation (HOPE) [118] enrolled 3,577 people with diabetes, who had a previous cardiovascular event or at least one other cardiovascular risk factor, were randomly assigned to ramipril (10 mg/day) or placebo. The combined primary outcome was myocardial infarction, stroke, or cardiovascular death. The study was stopped 6

months early (after 4.5 years) because of a consistent benefit of ramipril compared with placebo: ramipril lowered the risk of the combined primary outcome by 25% (95% CI 12-36, $p=0.0004$), stroke by 33% (10-50), and, among other outcomes, total mortality by 24% (8-37). After adjustment for the changes in SBP and DBP, ramipril still lowered the risk of the combined primary outcome by 25% (12-36, $p=0.0004$). The study demonstrated that the vasculoprotective effect of ramipril in diabetic patients was greater than that attributable to the decrease in blood pressure.

A secondary analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial, which tested the effect of treatment with atorvastatin in reducing stroke in subjects with a recent stroke or TIA, investigated the effects of treatment in subjects with type 2 DM or metabolic syndrome (MetS) [119]. In this subanalysis, subjects with type 2 DM ($n=794$) had increased risks of stroke (HR 1.62; 95% CI, 1.33-1.98; $p<0.001$), and major cardiovascular events (HR 1.66; 95% CI, 1.39-1.97; $P<0.001$) compared with patients with neither diabetes nor MetS ($n=3,295$). This exploratory analysis found no difference in the effect of statins in reducing these events in subjects with or without type 2 DM.

Intensive glucose therapy did not prove effective treatment in reducing the rate of cardiovascular events or death in patients with type 2 DM and prior history of cardiovascular disease, stroke, or vascular risk factors. In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial [12] [120], 10,251 diabetic patients were randomly assigned to intensive glucose control ($HbA_{1c}<6\%$) versus standard treatment ($HbA_{1c} 7-7.9\%$). The study was interrupted after 3.5 years of follow-up because of higher mortality in the intensive treatment group. No difference in rate of non-fatal stroke was observed between the two groups (HR 1.06; 95% CI 0.75-1.50; $p=0.72$). In the Action in Diabetes and Vascular Disease (ADVANCE) trial [121] 11,140 diabetic patients were randomized to intensive treatment ($HbA_{1c}\leq 6.5\%$) versus standard treatment ($HbA_{1c}\leq 7\%$). The two groups did not differ in occurrence of non-fatal stroke (HR 0.94; 95% CI 0.84-1.06; $p=0.32$). Also in the Veterans Affairs Diabetes Trial (VADT) trial [122] intensive glucose control did not reduce combined vascular outcomes compared to standard care (HR 1.07; 95% CI 0.81-1.42; $p=0.62$).

The PROspective pioglitAzone Clinical Trial in macroVascular Events (PROactive) was designed to evaluate efficacy of pioglitazone efficacy in preventing vascular events in patients with type 2 DM. In the subset of patients with history of stroke enrolled in the study ($n=486$ in the pioglitazone group and $n=498$ in the placebo group), pioglitazone was associated with a 47% RRR in recurrent fatal and non-fatal stroke (HR, 0.53; 95% CI, 0.34 to 0.85; $p=0.008$), and 25% RRR in stroke, MI, or vascular death (HR, 0.72; 95% CI, 0.53 to 1.00; $p=0.046$) [123].

Intensive hypertensive treatment (SBP <120 mmHg) in type 2 DM has not been supported by a recent analysis of the ACCORD patients. In this study the annual rate of the primary outcome (i.e., composite outcome was nonfatal myocardial infarction, nonfatal stroke, or death from cardiovascular causes) was 1.87% in the intensive BP treatment and 2.09% in the standard-therapy group (HR, 0.88; 95% CI 0.73 to 1.06; $p=0.20$). The annual rates of stroke were 0.32% and 0.53%, respectively (HR, 0.59; 95% CI, 0.39 to 0.89; $P=0.01$). Serious adverse events attributed to antihypertensive treatment were significantly more frequent in the intensive-therapy group (3.3% versus 1.3%; $p<0.001$) [120].

Conclusions: in patients type 2 DM with history of TIA and stroke, glucose control is recommended (Class I, Level of Evidence B) [124] [125]. Levels of $HbA_{1c} <6.5\%$ should not be achieved in diabetic patients with history of cardiovascular disease or vascular risk factors

[120] [121] [122]. Based on the current knowledge, a target BP <130/80 mmHg for patients with type 2 DM is recommended [124] [125]. An ongoing trial will provide further insight on pioglitazone usefulness in preventing recurrent stroke in diabetic patients.

ANTIPLATELET THERAPY

Within the established efficacy in stroke prevention through pharmacological and lifestyle control of modifiable vascular risk factors, a central role is played by antiplatelets. Aspirin represents the prototype of all antiplatelets. It acts by inhibiting the cyclo-oxygenase pathway with subsequent reduction in platelet thromboxane A₂ synthesis and partial block of the final step of platelet aggregation. In a recent meta-analysis of 16 secondary prevention trials (17,000 individuals at high average risk, 43,000 person-years, 3,306 serious vascular events) comparing long-term aspirin versus control, treatment with aspirin reduced of about a fifth the rate of total stroke (2.08% versus 2.54% per year, $p=0.002$), with a non-significant increase in haemorrhagic stroke [126]. Second generation platelet inhibitors such as thienopyridines (e.g., ticlopidine or clopidogrel) work by blocking the platelet adenosine diphosphate (ADP) receptor; they may offer greater preventive efficacy, especially in specific populations of atherothrombotic disease patients. In the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) study [127], over 19,000 patients at high risk of recurrent stroke were randomized to either treatment with clopidogrel 75 mg/day or aspirin 325 mg/day for a mean follow-up period of 1.9 years. Clopidogrel reduced the risk of major cardiovascular events (IS, MI, or vascular death) in a small but statistically significant manner compared with aspirin (RRR 8.7%; 95% CI, 0.3 to 16.5; $p=0.043$). However, sub-group analysis revealed that the RRR was statistically significant only in PAD but not in stroke nor in myocardial infarction (MI) patients.

In the Management of Atherothrombosis with Clopidogrel in High-risk patients with recent transient ischaemic attack or ischaemic stroke (MATCH) study [128] 7,599 patients were randomized to receive either clopidogrel 75 mg plus aspirin 75 mg or clopidogrel 75 mg alone. Combination therapy was not superior to clopidogrel alone in preventing primary composite outcomes (IS, MI, vascular death or rehospitalisation for any ischemic event), with significant increase in major bleeding complications. The Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial [129] evaluated whether the addition of clopidogrel to aspirin better prevented recurrent stroke. In this trial 15,603 patients with cardiovascular disease or multiple vascular risk factors for cardiovascular disease (35% had history of cerebrovascular diseases in the previous 5 years) were randomized to clopidogrel 75 mg plus low-dose aspirin (75-162 mg) or placebo plus aspirin (75-162 mg). The two groups did not differ in the rates of non-fatal IS (1.7% versus 2.1%; $p=0.07$) and had similar rates of intracerebral hemorrhage (0.3%). Patients in the combination arm treatment had a higher rate of moderate bleeding (not of severe or fatal). The study did not demonstrate superiority of combination treatment over aspirin in the subgroup of patients with prior history of IS or TIA.

Dipyridamole is a phosphodiesterase inhibitor which inhibits platelet aggregation. The European/Australasian Stroke Prevention in Reversible Ischemia Trial (ESPRIT) [130] was a randomized, non blinded study, comparing aspirin (30-325 mg) and dipyridamole (200 mg

twice daily; 83% extended-release dipyridamole) versus aspirin (30-325 mg) alone in 2,763 patients who had TIA, monocular blindness, and minor stroke (modified Rankin score ≤ 3) in the 6 months prior to enrollment. The mean follow-up was 3.5 years. Combined treatment conferred an absolute risk reduction of 1% for primary outcome (death from all vascular causes, nonfatal stroke, nonfatal myocardial infarction, or fatal bleeding complications). Despite few study weaknesses have been detected (non-blinded, nonstandard aspirin doses, divergence of significance between the on-treatment and intention-to-treat analyses, high rate of discontinuation therapy in the combination treatment arm), this study provided additional evidence of superiority of combined therapy with aspirin plus extended-release dipyridamole compared to aspirin alone in stroke prevention in patients with non-cardioembolic IS. Further, similar rates of recurrent stroke were observed with combination dipyridamole plus aspirin compared with clopidogrel, with no evidence that either of the two treatments was superior to the other in the prevention of recurrent stroke [131].

In the PERFORM study [132], the selective thromboxane-prostaglandin receptor antagonist terutroban (30 mg per day) was compared with aspirin (100 mg per day) in prevention of IS and cardiovascular events in patients with a recent non-cardioembolic IS. The primary efficacy endpoint was a composite of any IS, any MI, or other vascular death. The study was stopped prematurely for futility, because it showed similar rates of the primary endpoint with terutroban and aspirin, without safety advantages for terutroban.

Recommendations for patients with history of non cardioembolic stroke or TIA are: aspirin (50-325 mg/die) monotherapy (Class I, Level of Evidence A), the combination of aspirin 25 mg and extended-release dipyridamole 200 mg twice daily (Class I, Level of Evidence B), and clopidogrel 75 mg monotherapy (Class IIa, Level of Evidence B), are all acceptable options as initial therapy for prevention of recurrent stroke or other cardiovascular events. Clopidogrel is reasonable alternative option in patients allergic to aspirin (Class IIa, Level of Evidence C). The addition of aspirin to clopidogrel increases the risk of hemorrhage, and combination therapy is no recommended unless specific indications exist (i.e., coronary and other vascular stent or acute coronary syndrome) (Class III, Level of Evidence A). For patients who have an IS while on aspirin, alternative antiplatelets may be considered although this has not been assessed yet by RCTs (Class IIb, level of Evidence C) [74].

CILOSTAZOL IN THE MANAGEMENT OF ATHEROSCLEROSIS

Cilostazol is a selective and potent selective type III phosphodiesterase (PDE 3A) inhibitor that blocks cyclic adenosine monophosphate (cAMP) phosphodiesterase (PDE) in platelets and smooth muscle cells (SMCs), leading to inhibition of platelet aggregation and vasodilation, with potential use in atherosclerotic conditions, including stroke [133] [134]. Other properties of cilostazol are the inhibition of the uptake of adenosine, a platelet inhibitory agent, the suppression of platelet membrane glycoprotein P-selectin, which is involved in the interaction among platelets, leukocytes and vascular endothelial cells, responsible for the inflammatory and prothrombotic states induced by cerebral ischemia/reperfusion [134]. Cilostazol also has antiatherogenic properties: activates endothelial nitric oxide synthase (NOS) through its phosphorylation resulting in increased

nitric oxide (NO) release and angiogenic effect [135]; inhibits the expression of endothelial-neutrophil adhesion molecules mediated by high-glucose concentration, with potential application against vascular injury mediated by hyperglycemia [136]; increases lipoprotein lipase activity which is responsible for triglycerides hydrolyzation, reducing serum triglycerides and increasing HDL-C levels; inhibits the macrophage uptake of oxidized LDL by scavenger receptors (a major cellular event contributing to foam cell and fatty streak formation) [137].

Cilostazol efficacy has been tested in patients with periphery artery disease (PAD) with positive results in improving claudication [138]. A recent study evaluating the bleeding effects of platelet inhibitors (aspirin 325 mg/day, clopidogrel 75 mg/day and cilostazol 100 mg bid) each alone or in combination [139] [140], evidenced that the mean baseline bleeding time significantly increased after aspirin only, clopidogrel only, combination of aspirin and clopidogrel, and combination of all three agents; but not after cilostazol alone. The addition of cilostazol to aspirin or clopidogrel did not determine further significant increase in bleeding time compared to aspirin alone or clopidogrel alone.

The Cilostazol Stroke Prevention Study (CSPS) [141] [142] was designed to evaluate safety and effectiveness of cilostazol in prevention recurrent stroke. In this double-blind, placebo-controlled trial, 1,095 patients (544 receiving cilostazol 100 mg twice daily and 548 receiving placebo) were enrolled. The recurrence rate of IS was significantly reduced by cilostazol with a 41.7% RRR (number needed to treat=41). A subgroup analysis showed that patients with lacunar infarcts had a significant reduction in recurrence of IS whereas no statistical significance was reached in patients with atherothrombotic or mixed-type infarctions. The most commonly observed adverse event in the cilostazol treated group was headache (12.8%), palpitations (5.3%) and increase in pulse rate (19%). Headache and palpitations resulted in treatment discontinuation in some cases. This is in line with another PDE inhibitor, dipyridamole, currently used in stroke prevention, which caused withdrawal of therapy in 26% of patients in the ESPRIT trial [130].

Further potential use of cilostazol in clinical practice may derive from the benefits observed in diabetic patients. In patients with type 2 DM cilostazol 100-200 mg/day significantly prevented IMT progression, reduced the number of silent brain infarctions which have been associated with increased risk of developing dementia [143] [144]. Based on the results and the observed reduced efficacy of antiplatelets in diabetic patients [145], cilostazol might represent an alternative to standard care at least in the subgroup of patients with lacunar stroke. This hypothesis was tested in the Cilostazol versus Aspirin for Secondary Ischemic Stroke Prevention (CASISP) trial [146], which was a pilot, multicentre, double-blind trial, which randomly assigned 301 patients to cilostazol and 299 patients to aspirin treatment, with a follow-up period of 12 to 18 months. The primary endpoint was any recurrence of stroke (IS, haemorrhagic stroke, or subarachnoid hemorrhage) during the trial period, as assessed by follow-up MRI study. Cilostazol was associated with not-significant RRR of stroke (HR 0.62; 95% CI 0.30-1.26; $p=0.185$), and significantly lower rates of symptomatic and asymptomatic cerebral hemorrhages (7 vs 1, $p=0.034$). The results suggested that cilostazol appeared to be a more effective and safer alternative to aspirin in secondary stroke prevention, and that further trials were needed. Of interest, it should be further investigated whether a reduced risk of intracranial bleeding complications is peculiar of PDE inhibitors, as suggested by the reduced occurrence of cerebral hemorrhage observed in patients treated with dipyridamole plus aspirin as compared to aspirin alone in the ESPRIT [130]. In addition, a post-hoc analysis in patients

with cerebrovascular events enrolled in the CASTLE trial [147] detected a 3% absolute RR of cerebrovascular events compared to placebo (3.2% versus 6.1%; $p < 0.05$). This represents an important finding considering the 2-fold increased risk of cerebrovascular events in patients with PAD compared to patients without. Further, a RCT of 135 patients has shown that treatment with cilostazol significantly reduced progression of symptomatic intracranial arterial stenosis compared to placebo [148].

More recently, a systematic review assessed safety and effectiveness of cilostazol compared with aspirin in the prevention of stroke and other serious vascular events in patients with previous IS or TIA of arterial origin [149]. Two RCTs with 3,477 Asian participants were selected. Compared with aspirin, cilostazol was associated with a significantly lower risk of composite outcome of vascular events (6.77% versus 9.39%; RR 0.72, 95% CI, 0.57 to 0.91), haemorrhagic stroke (0.53% versus 2.01%, RR 0.26, 95% CI, 0.13 to 0.55), and minor adverse effects (8.22% versus 4.95%, RR 1.66, 95% CI 1.51 to 1.83).

Based on the available data, cilostazol seems to be more effective than aspirin in the prevention of vascular events in high-risk Asian patients. Further RCTs testing larger cohorts of vascular patients are needed in order to address the exact potential of cilostazol in the management of atherosclerosis and stroke prevention.

STROKE REHABILITATION

Stroke is one the leading causes of long-term disability in the developed countries. Its social impact is not only determined by the huge number of individuals that live with the effects of stroke, but there are also millions of relatives who care for stroke survivors and whose own lives are personally affected. According to the National Stroke Association 10% of stroke survivors recover almost completely, 25% recover with minor impairments, 40% experience moderate to severe impairments that require special care, 10% require care in a nursing home or other long-term facility, 15% die shortly after the stroke. Moreover, approximately 14% of stroke survivors experience a second stroke in the first year following a stroke [150].

A successful rehabilitation depends on several heterogeneous factors such as the amount of damage to the brain, the level of the rehabilitation team, the cooperation of family and friends, and the timing of rehabilitation. In fact, a crucial issue is that the earlier the rehabilitative programs begin, the more likely stroke patients can expect a more relevant clinical improvement. The goal of rehabilitation is to enable an individual who has experienced a stroke to reach the highest possible level of independence and be as productive as possible. Because stroke survivors often have complex rehabilitation needs, progress and recovery are unique for each person. Although a majority of functional abilities may be restored soon after a stroke, recovery is an ongoing process. The available scientific literature suggests that poststroke rehabilitation intervention is significantly more effective when it is delivered in the early phase of recovery (<6 months). Evidence supports that better functional outcome is determined by rehabilitation that is initiated promptly [151]. and based on intensive, especially multisensory, stimulation [152, 153]. This kind of stimulation is associated with increased adaptive plasticity of the brain in the early poststroke stages [154-157]. Indeed, optimal timing and dosing of rehabilitation interventions are yet unresolved

issues. For instance, high-intensity constraint-induced therapy (CIT), known to enhance activity in the lesioned hemisphere [158, 159], led to less improvement in upper extremity function compared to traditional therapy or low-intensity constraint-induced therapy, when administered early after stroke in the VECTORS study [160]. In this case, it has been proposed that an excessive disinhibition of the lesioned hemisphere that is induced very early after stroke may be harmful [161, 162].

Rehabilitative therapy should begin in the acute-care hospital after clinical stabilization, usually within 24 to 48 hours after the stroke. The first steps involve promoting independent movement because many individuals are paralyzed or seriously weakened. Depending on many factors—including the extent of the initial injury—patients may progress from sitting up and being moved between the bed and a chair to standing, bearing their own weight, and walking, with or without assistance. The neurological disabilities that are caused by an acute stroke mainly depend upon which area of the brain is damaged and how large is the lesion. However, as all stroke clinicians know, these symptoms may be extremely heterogeneous even if the lesions look quite similar. Generally, stroke can cause the following main types of disabilities: motor impairment; sensory disturbances including pain; aphasia; problems with thinking and memory; and emotional disturbances.

In a classic report, Twitchell described in detail the pattern of motor recovery following stroke. [150] At onset, the upper extremity (UE) is more involved than the lower extremity (LE), and eventual motor recovery in the UE is less than in the LE. The severity of UE weakness at onset and the timing of the return of movement in the hand are important predictors of eventual motor recovery in the UE. A systematic review of 58 studies confirms the most important predictive factor for upper limb recovery following stroke is the initial severity of motor impairment or function [163]. The prognosis for return of useful hand function is unfavorable when UE paralysis is complete at onset or grasp strength is not measurable by 4 weeks. However, about 9% of patients with severe UE weakness at onset may gain good recovery of hand function, and about 70% of patients showing some motor recovery in the hand by 4 weeks make a full or good recovery. Full recovery, when it occurs, usually is complete within 3 months of onset. Bard and Hirshberg asserted that if no initial motion is noticed during the first 3 weeks or if motion in one segment is not followed within a week by the appearance of motion in a second segment, the prognosis for recovery of full motion is not favorable. Although most recovery from stroke takes place in the first 3 months, and only minor additional measurable improvement occurs after the 6 months following onset, recovery may continue over a longer period of time in some patients who have significant partial return of voluntary movement [164, 165].

At least one-fourth of all stroke survivors experience language impairments, involving the ability to speak, write, and understand spoken and written language. A stroke-induced injury to any of the brain's language-control centers can severely impair verbal communication. Moreover, stroke can cause damage to parts of the brain responsible for memory, learning, and awareness. Stroke survivors may have dramatically shortened attention spans or may experience deficits in short-term memory. Individuals also may lose their ability to make plans, comprehend meaning, learn new tasks, or engage in other complex mental activities. Two fairly common deficits resulting from stroke are anosognosia, an inability to acknowledge the reality of the physical impairments resulting from stroke, and neglect, the loss of the ability to respond to objects or sensory stimuli located on the stroke-impaired side. Some emotional disturbances and personality changes are caused by the

physical effects of brain damage. Clinical depression appears to be the emotional disorder most commonly experienced by stroke survivors. Signs of clinical depression include sleep disturbances, a radical change in eating patterns that may lead to sudden weight loss or gain, lethargy, social withdrawal, irritability, fatigue, self-loathing, and suicidal thoughts. Post-stroke depression can be treated with antidepressant medications and psychological counseling.

All these complex syndromes can be accompanied by motor disorders and make the pathway toward a motor functional recovery more difficult and long.

NEUROPLASTICITY AND MECHANISMS OF RECOVERY

Brain plasticity is the ability of the nervous system to modify its structural and functional organization [166]. The most plausible forms of plasticity are collateral sprouting of new synaptic connections and unmasking of previously latent functional pathways. Other mechanisms of plasticity include assumption of function by undamaged, redundant neural pathways, reversibility from diaschisis, denervation supersensitivity, and regenerative proximal sprouting of transected neuronal axons. Experimental evidence indicates that plasticity can be altered by several external factors, including pharmacologic agents, electrical stimulation, and environmental stimulation. A key aspect of neuroplasticity that has important implications for rehabilitation is the fact that the modifications in neuronal networks are use-dependent. Animal experimental studies and clinical trials in humans have shown that forced use and functional training contribute to improved function. On the other hand, techniques that promote nonuse may inhibit recovery. In the past, the conventional wisdom was that benefits from rehabilitation were achieved primarily through training patients in new techniques that compensate for impairments (for example, using the uninvolved hand to achieve self-care independence). This approach avoided intense therapy on the weak upper limb. Currently, it is recognized that repeated participation by patients in active physical therapeutic programs probably provides direct influence on the process of functional reorganization in the brain and enhances neurologic recovery.

The discovery of the synaptic phenomenon of Long Term Potentiation (LTP) and Long Term Depression (LTD) demonstrated how the brain could adapt to encode novel experience at the level of the synapse or 'neuroplasticity' [166]. The most widely recognized forms of plasticity are learning, memory, and recovery from brain damage. At the synaptic level, plasticity induces dendritic spine formation, pruning, and remodeling, calcium channel regulation and changes in glutamatergic receptors. More recently, LTP and LTD of inhibitory GABAergic synapses have been described, and as is the case for glutamatergic synapses, both ionotropic and metabotropic mechanisms are involved. Many other neurotransmitters contribute to plasticity or its regulation, for example dopamine. While these mechanisms have been extensively investigated and clarified in animal models, only in recent years there have been attempts aimed to study how the human brain undergoes these crucial plastic changes [167]. A major step forward has been provided by using novel *in vivo* neurostimulation and neuroimaging techniques that have now allowed studying the same process in humans non-invasively. In particular, neurostimulation techniques like repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (TDCS) have been developed

to induce long-lasting changes in specific areas of the cerebral cortex that can be assessed with a high intra-individual reliability across different sessions, usually by means of motor evoked potentials (MEPs). rTMS protocols using patterned stimulation such as theta burst stimulation (TBS) have been more recently developed [168] using principles from tissue models and Hebbian models of plasticity, so that much lower intensities or shorter periods of stimulation are necessary to produce bidirectional LTP- and LTD-like changes in cortical excitability determined by changes of the MEP amplitude. Similar long lasting bidirectional changes can be obtained using tDCS [169]. Its primary mechanism of action involves changes in neuronal hyperpolarization, accomplished by cathodal tDCS and subthreshold neuronal depolarization, induced by anodal tDCS. The TBS and TDCS after-effects are NMDA receptor dependent [170] and affected by neuromodulators, such as dopamine, acetylcholine, and adrenaline [171]. Thus, the physiologic effects of TBS and tDCS show similarities to LTP and LTD, as induced in animals and in vitro brain slices. Crucially, these non-invasive brain stimulation (NIBS) techniques not only allow to investigate the mechanisms of cortical plasticity in healthy humans as well as in neuropsychiatric disorders, but the induction of neuroplasticity by means of NIBS is considered a promising tool in treating several neurological conditions [172, 173]. However, to be effective, these interventions must take into account the range of neurological disorders, their heterogeneity even within well-defined and characterized conditions, and the diverse time courses over which they act. Thus plasticity can be regarded as functional or dysfunctional, and this distinction is likely to be important for the application of NIBS in clinical situations. After brain lesions like stroke, cortical areas that are remote from the structural damage, such as the primary motor cortex, can reorganize to facilitate motor performance as well as motor learning [172, 173]. The available evidence suggests that cortical reorganization accompanies recovery of motor function after stroke. One form of cortical reorganization involves the modulation of interactions between the primary motor cortices in the ipsilesional hemisphere (same as the stroke), and the contralesional hemisphere [174, 175]. The influence of abnormal interhemispheric interactions on normal and abnormal cognitive functions after brain lesions has been reported in the fields of language and spatial orientation [176].

According to the model of interhemispheric competition, excessive inhibition of the lesioned hemisphere by the contralesional hemisphere may contribute to hand motor impairment in patients with mild motor deficits [174, 175]. Within this framework, rTMS has emerged as a potential tool to restore interhemispheric balance and improve hand motor performance. While high-frequency rTMS (HF-rTMS) often increases M1 excitability, low-frequency rTMS (LF-rTMS) has the opposite effect [173]. Up-regulation of excitability in the lesioned hemisphere by HF-rTMS or down-regulation of the contralesional hemisphere by LF-rTMS can facilitate movement of the paretic hand [173]. Both strategies, as well as their combination, have yielded encouraging results when applied to patients with mild hand motor impairment in the subacute and chronic stages after stroke [172].

Apart from NIBS methods, other strategies can be potentially useful to drive neuroplasticity and enhance mechanisms of recovery. Results from a systematic review indicate that modified constraint-induced movement therapy (CIMT) is more effective than traditional rehabilitation in reducing a patient's disability level [177]. It can improve upper extremity ability and increase movement spontaneity. Further studies are needed on CIMT's effectiveness in kinematic analysis. In a pilot, randomized, clinical trial, with a 6-month follow-up, the practicality and efficacy of conventional neurological therapy, constraint-

induced therapy, and therapeutic climbing to improve minimal-to-moderate arm and hand function in stroke patients was evaluated. The study concluded that improvement of arm and hand function in the intermediate term was best achieved using the constraint-induced therapy approach [178].

The use of robotic systems to complement standard poststroke multidisciplinary programs is a recent approach that looks very promising; robotic devices can provide high-intensity, repetitive, task-specific, interactive treatment of the impaired limb (passive and/or active-assisted exercises) and can objectively and reliably monitor patients' motor progress, measuring changes in movement kinematics and forces [179, 180]. It is appropriate to consider the robot as an advanced tool to be used under the therapist's direction—a tool that can implement relatively simple repetitive and labor-intensive therapies. Clinical decisions should be managed by the rehabilitation team and, when appropriate, planned and executed on the robot; this approach should be part of an integrated set of tools that also includes simpler nonrobotic approaches [181].

In a recent Cochrane review, Mehrholz et al. showed that the use of electromechanical devices in rehabilitation may not significantly improve activities of daily living (ADLs), although they did find evidence that upper-arm motor function and strength may improve [182]. Moreover, a recent multicenter, randomized, controlled trial involving 127 patients with moderate-to-severe upper-limb impairment 6 months or more after a stroke, was performed in order to verify the potential of Robot-assisted therapy for long-term upper-limb impairment after stroke. In patients with long-term upper-limb deficits after stroke, robot-assisted therapy did not significantly improve motor function at 12 weeks, as compared with usual care or intensive therapy, although in secondary analyses, robot-assisted therapy improved outcomes over 36 weeks as compared with usual care but not with intensive therapy [183]. Thus, the actual role of robot-assisted therapy in poststroke rehabilitation remains to be clarified, and to date, no guidelines exist on how the design and use of such devices might increase their efficacy [182].

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Chapter 3

ISCHEMIC STROKE PREVENTION IN VIETNAM

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ABSTRACT

Ischemic stroke has a high burden not only in developed countries but also in developing countries. In Vietnam, rapid economic development and modernization during the past two decades has led to dramatic increases in many chronic diseases, including ischemic stroke. A previous study of stroke conducted in the southern region of Vietnam reported an age-adjusted prevalence of 608 per 100,000 people, while mortality and incidence of the disease were estimated to be about 131 and 250 per 100,000 people, respectively. Hypertension was identified as one of the most important risk factors. However, recent epidemiologic information about ischemic stroke for the Vietnamese population is lacking. Unlike non-modifiable factors (e.g., age, gender, race and genetic predisposition), modifiable and potentially modifiable lifestyle-related factors such as tobacco smoking, alcohol consumption, poor diet, physical inactivity, overweight and obesity, have been recognised as vital for both primary and secondary prevention in the literature. The purpose of this chapter is to review the epidemiology and risk factors of ischemic stroke, with implications on the prevention of this emerging chronic disease in Vietnam.

INTRODUCTION

Strokes are caused by disruption of the blood supply to the brain. This may result from either blockage (ischemic stroke) or rupture of a blood vessel (haemorrhagic stroke) [1]. Each year about 795 000 people experience a new or recurrent stroke. About 610 000 of these are

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first attacks, and 185 000 are recurrent attacks [2]. Stroke is a global epidemic, a health problem not limited to Western or high-income countries. Actually, about 85% of all stroke deaths are registered in low- and middle-income countries, which also account for 87% of total losses due to stroke in terms of disability-adjusted life years [3]. Stroke incurs a huge burden to the society. The treatment and productivity losses due to death and disability from stroke lead to large societal costs and healthcare expenditure in many countries.

STROKE PREVALENCE AND INCIDENCE

The prevalence of stroke varies by sex and regions, partly due to different methods of estimation between studies [4]. A review of 23 data sources indicated stroke is becoming more common worldwide [5]. In developing countries, the stroke prevalence is increasing significantly. For example, the standardized stroke prevalence among Chinese adults aged 35 years old and above was 1111.5 per 100,000, yet the rate of urban residents (1544.8 per 100,000) was twice the rate of those living in rural areas (758.1 per 100,000) [6]. A study in Bangladesh involving 15627 participants aged 40 years and above found a higher stroke prevalence among men than women, the male to female ratio being 3.44:2.41 per 1000 [7], while twocommunity-based studies conducted in the Philippines reported a prevalence ranging from 1.9% to 6.5% [8]. In Vietnam, little data on stroke prevalence are available. A previous study conducted during 1994-1995 in the southern region of Vietnam reported an age-adjusted prevalence of 608 per 100,000 people, while mortality and incidence of the disease were estimated to be about 131 and 250 per 100,000 people, respectively [9].

MORBIDITY AND MORTALITY TREND

A systematic review showed a divergent, statistically significant trend in stroke incidence rates over the past four decades, with a 42% decrease in stroke incidence in high-income countries but a greater than 100% increase in stroke incidence in low to middle income countries. In 2000-2008, the overall stroke incidence rates in low to middle income countries have, for the first time, exceeded the level of stroke incidence seen in high-income countries by 20% [10]. During the past three decades, transition has taken place in many developing countries in terms of economy, demography, lifestyle, and health [11]. Non-communicable diseases (NCD) such as cardiovascular disease and stroke have emerged as leading causes of morbidity and mortality [12]. In Vietnam, rapid economic development and modernization have taken place since 1990's. Similar to other developing countries, Vietnam is also experiencing fast transition resulting in a widening gap between rich and poor people, degradation of the environment, an ageing population, and increasingly unhealthy lifestyles.

Consequently, Vietnam is now facing a “double burden” of old communicable diseases and emerging NCD. According to hospital statistics for the whole country, NCD admissions rose from 39% in 1986 to 65% in 1997 and cases of death from NCD increased from 42% in 1986 to 62% in 1997 [13]. Ischemic stroke was ranked number one among the leading causes of death [14].

RISK FACTORS OF STROKE

Stroke is not a single disease but a highly complex syndrome consisting of a group of heterogeneous disorders with many genetic and environmental risk factors [15, 16]. Risk factors for a first stroke are classified according to potential for modification (non-modifiable, modifiable, or potentially modifiable) and strength of evidence (well documented or less well documented) [17].

Non-Modifiable Risk Factors

Non-modifiable risk factors include age, gender, low birth weight, race/ethnicity, and genetic predisposition. In particular, gender plays an important role in the epidemiology of stroke. Worldwide, stroke is more common among men, but women are more severely ill. A review assessing 59 incidence studies from 19 countries and 5 continents claimed that male stroke incidence rate was 33% higher and stroke prevalence was 41% higher than those of the females, with large variations between age bands and between populations. However, stroke tends to be more severe in women, with a one-month case fatality of 24.7% compared to 19.7% for men [18].

Age is another well-known non-modifiable risk factor for stroke. In a prospective study, age at natural menopause before age 42 was associated with an increased ischemic stroke risk. Women with menopause before age 42 had twice the stroke risk compared to all other women [19].

On the other hand, studies on twins, family history and animal models [20-22] provide evidence for genetic contribution to the common forms of stroke, yet no major risk variants have been identified showing consistent results across populations.

Modifiable Risk Factors

There are many well-documented and modifiable risk factors for ischemic stroke. These include hypertension, exposure to cigarette smoke, alcohol consumption, diabetes, atrial fibrillation and certain other cardiac conditions, dyslipidemia, carotid artery stenosis, sickle cell disease, postmenopausal hormone therapy, poor diet, physical inactivity, and obesity and body fat distribution.

In relation to smoking, a study reported the relative risk increases from 1.16 for exposure to five cigarettes per day to 1.56 for exposure to 40 cigarettes per day. There is evidence of a strong, consistent and dose-dependent association between exposure to smoking and risk of stroke, suggestive of a causal relationship, with disproportionately high risk at low levels of cigarette smoking suggesting no safe lower limit of exposure [23].

For alcohol consumption, dose-response analysis revealed that the lowest risk of stroke mortality occurred with at most one standard drink per day. Secondary analysis of mortality from all causes showed lower risk for drinkers compared to non-drinkers. Consequently, light to moderate alcohol consumption is associated with a reduced risk of stroke [24].

An epidemiological survey in China found the stroke incidence significantly higher among individuals with atrial fibrillation (12.1%) compared with those without (2.1%) [25]. The prevalence of stroke in nonvalvularatrial fibrillation patients was 24.2%, and this rate increased dramatically with age [26]. For example, the prevalence of stroke was 4.3% in patients younger than 40 years of age but increased to 32.9% among atrial fibrillation patients 80 years of age or older. Therefore, atrial fibrillation is considered a major risk factor for ischemic stroke [26].

In relation to dietary pattern, a Chinese study concluded that the Western diet characterized by high consumptions of beef, fruit, egg, poultry, and seafood is associated with an elevated risk of stroke, but the associations become non-significant after adjustment for obesity, hypertension, hyperglycemia, and dyslipidemia [27]. Indeed, the combination of the four risk factors, namely, hypertension, diabetes, obesity and cigarette smoking, conferred the highest odds of having an cerebrovascular event [28].

Physical inactivity or a sedentary lifestyle also contributes to the stroke etiology. A recent large cohort study observed that moderate- to heavy-intensity physical activity, but not energy expended, is protective against ischemic stroke independent of other stroke risk factors in men [29].

It is known that treatment with cholesterol-lowering medications and changes in low-density lipoprotein cholesterol level over time may attenuate the stroke risk, while the role of lipids in the development of ischemic stroke has been less consistently observed [30].

Other modifiable risk factors for stroke examined in the literature include sleep duration [31], diabetes [32], exposure to antipsychotics [33], and low serum magnesium level [34]. In view of the large number of plausible risk factors, tackling any single lifestyle related factor is therefore not sufficient for the primary and secondary prevention of ischemic stroke [35].

PREVENTION STRATEGY IN VIETNAM

Stroke is a severely neglected problem in lower income countries such as Vietnam. Population-based epidemiological studies and stroke prevention programmes to inform and control stroke are urgently required. On the basis of successful experiences in cardiovascular disease, such studies should now be feasible in stroke [36].

In particular, large-scale epidemiological studies that utilise standardised approaches to measure risk factor exposures for each stroke subtypes are needed to understand the reasons for differences between regions and subpopulations, which have implications for the development of population-based prevention programmes. Large samples are also required in such studies to distinguish interactions between risk factors and exposures (e.g., between genes and environment).

Current approaches to primary preventive action emphasize the need to target the absolute risk of disease rather than individual risk factors. Lifestyle interventions serve as a basis for primary prevention of ischemic stroke. It is estimated that 70%of strokes are potentially preventable by lifestyle modification, but prospective evidence is required to support these hypotheses derived from epidemiological studies. Different strategies for drug interventions in primary prevention have been proposed, including the polypill strategy.

Based on the available evidence, additional preventive measures should focus on blood pressure, chronic heart failure, and possibly lipids [37].

In Vietnam, a study was conducted during 2001-2009 in the rural areas of Ba-Vi district, Ha-Tay province [38]. It concluded that alarming increases in risk factors in the general population warrant comprehensive multi-level prevention strategies. Both top down and bottom up approaches were applied to implement a hypertension management programme in the rural commune. The study demonstrated the feasibility, applicability and benefit of a community-based intervention in a low resource setting to engage all involved partners. This strategy should be incorporated as part of an integrated and coordinated national programme to prevent and control ischemic stroke [38].

CONCLUSION

Ischemic stroke is diversely reported by different countries and regions worldwide. Although a slight declining trend has been observed in the US and some European countries, in low and middle-income countries, the rate of ischemic stroke is increasing rapidly. There are multiple risk factors for stroke in these countries due to poor management and less effective control programmes. In Vietnam, epidemiological information about ischemic stroke for the population is currently lacking. Further studies are needed to provide evidence for the development of an effective prevention and control programme in the future.

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Chapter 4

MODULATION OF PROTEIN KINASE C ISOFORMS: A POTENTIAL THERAPEUTIC FOR ISCHEMIC STROKE?

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ABSTRACT

Manipulation of the protein kinase C (PKC) signaling pathway has been recognized as a potential therapeutic target for numerous disease states, the most well-investigated being cardiovascular disease. Recent discoveries in preclinical models of neurologic disease ranging from Alzheimer's disease (AD) to traumatic brain injury (TBI) to stroke have identified changes in PKC expression and in some cases, successfully targeted this pathway therapeutically. The effects of altered PKC expression following injury remain under investigation with some studies implicating PKC in the regulation of oxidative stress, apoptosis, inflammation, and vascular remodeling. Additionally, various PKC isoforms may be involved in both beneficial and detrimental processes following injury as evident by the role of PKC in mediating neuroprotection following hypoxic preconditioning but also being associated with blood-brain barrier (BBB) disruption in other studies. Furthermore, preclinical studies of neurologic injury and disease often fail to emphasize the effect of aging, despite the fact that age is the greatest risk factor for numerous neurologic diseases such as stroke and AD. This is of particular relevance in

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the regulation of PKC function as aging has been shown to alter enzyme translocation, thus altering function. This work reviews the location and pathways associated with various PKC isoforms, the role of PKC isoforms in the aging process, as well as implications of PKC signaling following neurologic or cerebrovascular injury. We provide insight into potential downstream pathways of interest and the potential role of PKC modulation as a therapeutic target for ischemic stroke based on findings in not only preclinical studies of stroke but also studies of other neurologic and cardiovascular diseases.

INTRODUCTION

PKC Isoforms – A Lesson in Structure-Function Relationships

The PKC family is a series of threonine/serine kinases. Up to twelve different isoforms exist each with their own actions and specific pathways of activation. All isoforms are present in some form or another within the brain [1]. These isoforms have been grouped into three classifications: conventional, novel, and atypical. The classification is based on the unique polypeptide chains and the cofactors associated with each group. The NH₂-domain is specific for the membrane phospholipids that interact with PKC during translocation.

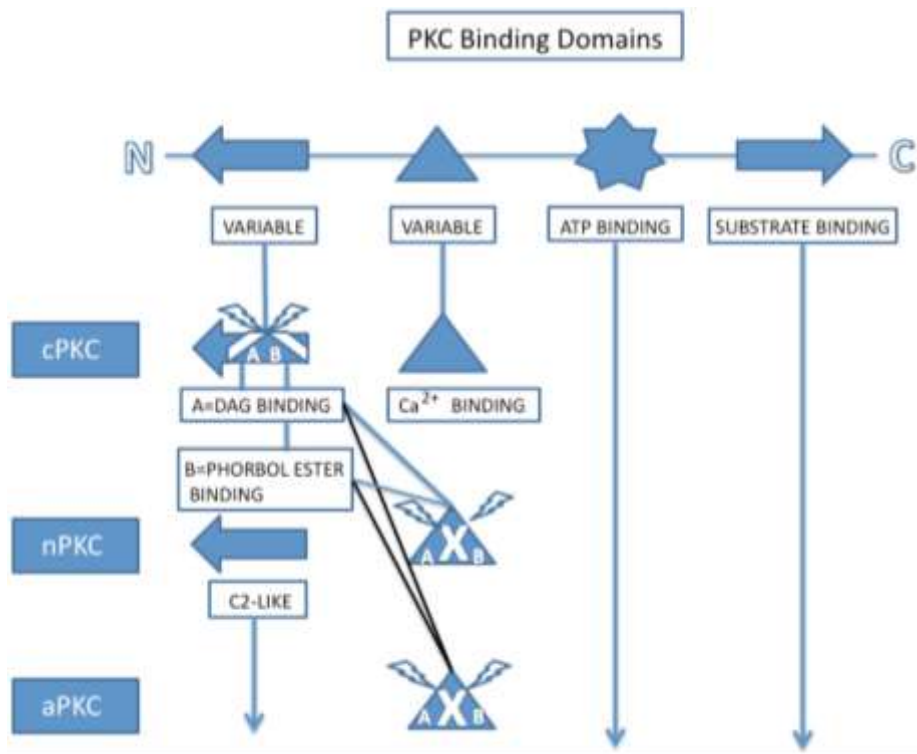


Figure 1. Schematic of typical protein sequence for PKC showing the variations between conventional, novel, and atypical subgroups. cPKC requires diacylglycerol, phorbol ester, and calcium for activation. nPKC requires diacylglycerol and phorbol ester but not calcium for activation. aPKC is not dependent on diacylglycerol or phorbol ester but activates quicker in their presence which is represented by the black lines.

The COOH-domain is specific for the activating cofactors, and the C2 domain has binding capacity for receptors for activated C kinases (RACKs). Conventional isoforms (cPKC α , - β I, - β II, and γ) require calcium, phosphatidylserine (PS), and diacylglycerol (DAG) for activation. The novel isoforms (nPKC δ , - ϵ , - η , - θ , and - μ) require PS and DAG but not calcium.

The atypical isoforms (aPKC ζ , - λ) are unresponsive to DAG, PS, and calcium (Figure 1). Both cPKCs and nPKCs activate the extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) cascade among other pathways [2]. These pathways will be discussed in detail in later sections.

In investigating PKCs, it was discovered that a panel of nine genes express the phospholipid-kinases. These genes produce the PKCs in various tissues throughout the body and account for their broad distribution and functions. PKC γ , PKC δ , PKC α , and PKC ϵ are the most predominant isoforms associated with neurologic diseases. Other isoforms play more predominant roles in other organ systems such as the heart.

Expression of PKC Isoforms – Systemic and Cellular Considerations

PKCs have been found in a large number of organs such as the heart, brain, lungs, kidneys, and organs of the gastrointestinal tract as well as in cells of the hematopoietic system [3-6]. Different isoforms are located in a tissue-specific manner. PKC α is highly expressed in β cells of the pancreas, but has also been linked to pathological tissue such as breast cancer and malignant gliomas [7]. PKC γ is endogenous to the α cells in the pancreas. PKC ζ has been linked to matrix metalloproteinase (MMP) regulation in smooth muscle cells [8]. PKC δ is upregulated in endothelial cells following ischemia [9]. PKC ϵ is present in large quantities in the hippocampus and olfactory tubercle [10]. PKC β II is present in both normal and pathological B-cells [11]. Other isoforms such as PKC θ and PKC α work together and have important roles in T-cell regulation and IL-2 activation [12]. All PKCs are originally localized in the cytosol of specific cells and must undergo a translocation to a membranous site. Depending on the isoform or tissue, this membranous site could be the nucleus, golgi apparatus, endoplasmic reticulum, mitochondria, or even the cell membrane [13, 14].

Activation of PKC Isoforms

PKCs are completely phosphorylated following translation but are inactive due to pseudosubstrate interference with the binding domain. The pseudosubstrate domain keeps the PKC polypeptide in a folded configuration. PKCs are localized in the cytosol of specific cells and must be activated by removing the pseudosubstrate domain. After activation, PKCs must then be translocated to membranous sites in order to phosphorylate downstream pathways [15]. Lipopolysaccharide (LPS) in the presence of the aforementioned endogenous cofactors (DAG, Ca⁺⁺, and PS) induces activation of PKC β , - ϵ , and - ζ [16]. Phorbol esters such as tissue plasminogen activator (TPA) and even the β -adrenergic system have also been shown to activate PKCs [17]. A variety of growth factor receptors can activate PKCs as well. The increase in growth factors during cancer is one of the reasons PKCs have become ideal targets for therapeutic interventions [18]. Once activated, PKCs can be translocated. The

translocation mechanism is initiated through PKC binding with RACKs. RACKs are 30-36 kilo-Dalton proteins that are located in cytoskeletal compartments. They interact with a C2 domain on the PKC polypeptide and thus do not interfere with the substrates binding site. Each isoform of PKC has a specific RACK. RACKs also have other functions besides binding PKCs such as anchoring phosphodiesterase and integrin, and contributing to vesicle release and cell-to-cell communication [19, 20].

After PKC binds to its isoform specific RACK, the entire unit will translocate from the cytoplasm to the membranous sites previously mentioned.

Regulating PKC Activation

In general, PKCs are self-regulated proteins. When PKCs are activated, the pseudosubstrate dissociates. Eventually, the PKC loses one of its three phosphate groups at a site known as the activation loop. When this occurs, the cofactor separates from the active site and the PKC is no longer functionally active.

The pseudosubstrate reattaches and the PKC is in its inactive state. In order for the PKC to return to a state where it can activate again, it must regain the phosphate group that it has lost. A phosphoinositide-dependent kinase 1 (PDK-1) phosphorylates the PKC at the activation loop. This additional phosphorylation causes the other remaining phosphate groups to reconfigure and realign. In some PKCs, PDK-1 does not directly phosphorylate the activation loop but instead induces autophosphorylation. Once all phosphate groups have been restored and the other phosphate groups have been reconfigured, the PKC can undergo activation [21].

PKC – A Role in Disease Processes?

PKCs typically regulate important functions such as cell growth and differentiation, secretion of hormones, and normal membrane functioning. They also play a key role in the regulation of the Na/K/2Cl-cotransporter (NKCC) and neuronal sodium and potassium transport. Several of the PKC isoforms contribute to the pathology of neurologic and cardiovascular disease sometimes in direct contrast to one another. In stroke, the PKC isoforms α , β I, and ϵ contribute to the activation of NKCC at the blood-brain-barrier (BBB). This leads to the protectant mechanism of increased K^+ transport across endothelial cells and an increased K^+ flow back into the blood stream [22]. PKC β I also acts as a neuroprotectant for astrocytes by inducing phosphorylation and increasing the activation of excitatory neurotransmitter transporters. Similarly, PKC α acts as a neuroprotectant for neurons by activating the adenosine signaling pathway [23]. In contrast to these neuroprotectant roles, PKC δ in conjunction with amplified Ca^{++} influx has been shown to cause increased constriction of the middle cerebral artery (MCA) prior to and during ischemic stroke [9, 24]. Following ischemia, PKC β II has been linked to increased endothelial permeability and inflammation [25]. Stroke is not the only neurologic disease where PKCs are active.

The isoform PKC δ also regulates mitochondrial-induced apoptosis and has been linked to diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Amyotrophic Lateral

Sclerosis (ALS), and Traumatic Brain Injury (TBI) [26-29]. PKC δ is cleaved by caspase-3 and translocates to specific cells via the appropriate RACK. It then enters the mitochondria of these target cells, and eventually phosphorylates a series of enzymes such as the GTPase Drp1.

This signaling cascade ultimately leads to mitochondrial-mediated apoptosis [30]. PKCs also play a critical role in cardiovascular disease. PKC ϵ provides cardioprotection through activation of mitochondrial aldehyde dehydrogenase 2 (ALDH2). PKC ϵ is originally found in the cytosol and translocates to the cell particulate fraction following hypoxia preconditioning. In addition to an interaction with the appropriate RACK, PKC ϵ associates with heat shock protein 90 (HSP90) before it translocates [31]. Similar to the brain, PKC δ leads to reperfusion injury following the initial onset of myocardial infarction. Importantly, cardiovascular-based studies have revealed an increase in PKC translocation by over 50% following injury. When inhibited, injury is significantly reduced through attenuation of the pro-apoptotic pathway in mitochondria [32].

If an animal is pre-conditioned to ischemia before injury, the protectant isoform PKC ϵ will be upregulated and the harmful isoform PKC δ will be downregulated [33, 34]. As more is discovered about PKCs, it is becoming increasingly apparent that they have important functions in a broad spectrum of diseases.

This is particularly true when considering the greatest risk factor for the development of neurologic disease is aging. Since age in and of itself alters the dynamics of PKC signaling, it is important to realize that unique modifications occur in aged animals even before the onset of disease [35].

EFFECT OF NEURAL DISEASE AND INJURY ON PKC ACTIVITY

Numerous disease states, both neurologic and non-neurologic in nature, have been associated with changes in both PKC isozyme activity and translocation. The following sections highlight the role of common neurologic disease states in modulation of PKC activity but it's important to consider that much of what is known concerning the role of PKC in pathophysiology is derived from work in other organ systems, namely cardiovascular.

Many comprehensive reviews focusing on the general role and effects of PKC following injury, as well as specific isozyme-mediated effects, have been published by the Mochly-Rosen group and others, often in reference to cardiovascular disease, a disease process that likely shares many similarities with cerebral ischemia. As such, the cardiovascular literature and the aforementioned reviews provide insight into the role of PKC in neural injury and can serve as further reference for material not covered herein [36].

The interest in PKC isozyme modulation following neurologic injury appears warranted due to the identification of deficits and abnormalities in PKC-mediated signaling cascades, particularly in relation to learning and memory deficits, in the brain that occur acutely in patients with Alzheimer's disease and/or ischemic stroke [37]. Of particular interest is the role of the epsilon izoyme as PKC ϵ is expressed more highly in the brain than most other tissue, suggesting a critical role in nervous system homeostasis. This notion is consistent with the role of PKC ϵ in various functions including perhaps most notably, neurotransmitter release and ion channel function [10]. Furthermore, contrasting viewpoints concerning the effect of

neural injury on PKC activity exist and therefore, additional investigation is required to understand both these effects and how PKC can be manipulated pharmacologically for therapeutic benefit [3].

Alzheimer's Disease (AD)

Of the neurologic disease states in which PKC has been investigated in relationship to disease pathophysiology, AD comprises the vast majority of reported studies. Importantly, deficient PKC isozyme function has been described as a key component of disease pathogenesis and consequently raised the prospect of PKC as a promising therapeutic target [38]. Pathologic studies conducted on the brains of AD patients reveal a close correlation between dementia and synaptic loss. The precise mechanisms leading to synaptic loss have been poorly elucidated until recent work of Hongpaisan, et al. in which a reduction in synaptogenic PKC isozymes, particularly α and ϵ , and associated substrates was found to occur in conjunction with increased soluble beta amyloid but prior to both plaque development and corresponding neuronal loss. These findings were consistent with clinical autopsy results in which age-matched, non-demented control patients exhibited a higher level of PKC expression and activity in comparison to patients afflicted with AD [39].

Mechanistic studies have revealed a role for PKC activation in activation of α -secretase, an enzyme responsible for converting amyloid precursor protein (APP) to soluble sAPP α , potentially preventing amyloid beta (A β) production. Besides altering production of A β , PKC-mediated pathways have been reported to increase the degradation of A β , further altering the ratio of production and degradation in a beneficial manner [40]. In addition to providing a potential therapeutic approach to AD, these studies have also demonstrated clear roles for many PKC isozymes in learning and memory-associated tasks that are affected in many neurologic disease states such as ischemia and traumatic brain injury [38].

Ischemia

Conflicting evidence exists concerning the effect of ischemia on PKC expression and activity [2, 3, 25, 41, 42]. This is thoroughly reviewed by Bright and colleagues and perhaps most importantly, only recently have the effects of various isozymes been recognized as frequently divergent, necessitating the consideration of disease-isozyme relationships rather than consideration of PKC activity/expression as a whole [1]. We attempt to identify these often different, if not opposing, roles of various isozymes as related to disease pathophysiology and therapeutic development in the remainder of this work.

In addition to consideration of potentially contrasting isozyme effects, the impact of preclinical model and corresponding severity of ischemia has often gone unrecognized. Importantly, Sun and colleagues investigated this precise issue, demonstrating that while PKC activity is increased with mild ischemia, severe ischemia results in diminished PKC activity [37]. Besides severity of ischemia, the location of both ischemia and sample must be taken into account. Cortical and striatal tissue may respond differently to ischemia due to unique metabolic demands and cellular composition. Whether tissue samples analyzed are from

infarct core or penumbral regions may further influence PKC-related findings [43]. Other factors to consider include the time of analysis post-onset of ischemia, the role of isozyme phosphorylation in activation and downstream-signaling, and discrepancies between *in vitro* and *in vivo* studies. Contrasting results between cell-based and animal-based studies, as reported by Fleegal and colleagues, indicate the potential for cell-specific effects, further complicating the study of PKC-mediated pathways [25]. Whether manipulation of PKC in response to injury has similar effects across the various cell-types found within the brain remains to be seen.

Regardless of the aforementioned issues in comparing results across studies, it is clear that PKC is involved in both the initial, acute response to ischemia as well as more delayed effects throughout the subacute and chronic periods. This is consistent with work showing PKC δ increased throughout both infarct core and penumbra regions [37]. Likewise, PKC ϵ has been shown to be up-regulated rapidly following onset of ischemia and remains elevated in both *in vitro* and *in vivo* studies [2, 44]. Most importantly, preclinical studies targeting specific PKC isozymes using either pharmacologic agents or genetic modifications have demonstrated the ability to reduce cerebral injury [1, 45, 46].

Ischemic Preconditioning

In addition to the effect of ischemia on PKC and modulation of PKC for therapeutic benefit discussed above, PKC is also involved in the beneficial effects associated with ischemic preconditioning [10, 37, 47]. Historically, the PKC ϵ isozyme has been most frequently recognized as essential in ischemic preconditioning [10, 37, 48]. The protective effects associated with PKC ϵ activation that occurs with preconditioning have been ascribed to the role of PKC ϵ in the adenosine/NMDA-activated signal transduction pathway [37]. Recent work in mice undergoing hypoxic preconditioning (HPC) prior to middle cerebral artery occlusion (MCAO) has implicated PKC β II.

This finding is consistent with observed increases in PKC β II membrane translocation in the acute phase of HPC, but further work remains to clearly elucidate the mechanism of protection [47, 48]. Other isozymes such as PKC δ , a pro-apoptotic kinase, have been identified as being decreased with HPC in cardiac tissue, which may lead to the protective effects associated with HPC [34].

Traumatic Brain Injury

Relatively little work has been done investigating the effect of TBI on PKC activity with the exception of that by Yang and colleagues and Zohar and colleagues. Yang, et al. demonstrated significant changes in PKC activity at 1 and 3 hours after TBI but not at 5 minutes or 24 hours post-injury. At the 3 hour time point, PKC translocation to membrane fractions was elevated [3]. While Zohar, et al. did not assess activity or translocation, administration of bryostatin-1, a potent PKC activator, resulted in diminished learning and memory deficits following TBI. The reduction in learning and memory deficits was consistent

with improved maintenance of synapses as evident by pre-synaptic synaptophysin and post-synaptic spinophylin immunohistochemistry [27].

ROLE OF PKC IN NEURAL INJURY OUTCOME

The effect of altered PKC activity observed in many of the aforementioned neurologic disease and injury states has not been entirely elucidated but many recent works have tied various PKC isozymes to oxidative stress, regulation of apoptosis, blood-brain barrier (BBB) breakdown, and processes associated with recovery/repair. In the following sections we expand upon work investigating the role of PKC in these disease-associated events by again linking work completed across multiple organ systems, mainly neurologic and cardiovascular.

Modulation of Response to Oxidative Stress

Oxidative stress has frequently been cited as a potentially precipitating factor in many pathologic states, particularly those associated with the aging process resulting in the theory of ‘inflammaging’ [49]. A reduction or loss of PKC activity has previously been correlated with injury severity but how PKC modulated this response was unclear. Emerging evidence implicates PKC in the modulation of oxidative stress-induced apoptosis, generation of oxidative stress through NADPH oxidase, and mitochondrial function.

Oxidative stress leads to PKC δ translocation to the mitochondria, termed mitochondrial targeting, ultimately causing loss of mitochondrial transmembrane potential and release of cytochrome c that leads to apoptosis [32, 50]. PKC δ also appears to be somewhat unique in the response to oxidative stress in that it may also further amplify the apoptotic cascade via caspase activation and subsequent feedback [51]. Importantly, previous work has shown these processes can be inhibited using PKC δ inhibitors [50, 52]. These inhibitors may also prevent upregulation of NOX1, part of the NADPH oxidase complex, via PKC δ that contributes to oxidative stress and has been identified previously as a potential therapeutic target [53-55].

Effect of PKC on Cell Survival

Consistent with divergent effects of PKC isozymes on other cellular functions, PKC isozymes have been characterized as pro- or anti-apoptotic in nature. Consequently, work utilizing non-specific PKC modulators has shown both protective and deleterious effects in response to injury, likely due to isozyme-specific effects [56]. PKC isozymes identified as suppressing apoptosis include α , β , ϵ , and ζ while those acting as pro-apoptotic are δ and θ .³⁷ Most work investigating PKC-mediated effects on cell survival, namely apoptosis, has centered on the role of PKC δ and PKC ϵ .

Inhibition of PKC δ has been found to be universally protective regardless of organ system following onset of ischemia as evident by diminished pathologic features of apoptotic processes such as DNA laddering and fragmentation [32, 57]. Similarly, proteins involved in apoptotic signaling cascades such as cytochrome c, caspase 3, and Bcl-2 associated death

protein (BAD) were reduced following inhibition [32, 57-60]. Perhaps most importantly, at least from a therapeutic development standpoint, is that administration of PKC δ inhibitors results in improved outcome following ischemic stroke, even when administered up to 6 hours after onset [58].

How exactly PKC δ mediates the aforementioned effects remains to be clearly elucidated but numerous pathways have been proposed in which glycogen synthase kinase-3B (GSK-3B) and Akt signaling pathways are altered [57, 58]. Others have shown a role for Drp1, a mitochondrial fission protein to which PKC δ binds, that is phosphorylated upon binding resulting in enhanced mitochondrial fragmentation [59]. Furthermore, PKC δ may be partially responsible for mediating the protective effects of hypothermia following ischemia [41].

Investigation of PKC ϵ is more limited and the effects not as clearly elucidated as those associated with PKC δ modulation. Bright and colleagues demonstrated a protective role of $\psi\epsilon$ RACK and associated PKC ϵ -mediated effects when activator peptide is administered near the time of stroke onset, implicating PKC ϵ in acute pathophysiologic events. When given 24 hours before ischemia or at the onset of reperfusion, $\psi\epsilon$ RACK-mediated activation did not improve nor worsen outcome [44]. In other work, activation of PKC ϵ restores the phosphorylation state of endothelial nitric oxide synthase (eNOS), increasing cell survival [61].

Regulating BBB Integrity Through Matrix Remodeling

Neural injury and degeneration often involves transient periods of blood-brain barrier (BBB) disruption. Improving the maintenance of BBB integrity has been identified as a promising therapeutic target for neurologic disease, particularly ischemic stroke. Much of the previous work into mechanism of BBB breakdown after injury has focused on the role matrix metalloproteinases (MMPs) play in both acute breakdown, associated with deleterious processes, as well as chronic regulation required for recovery/repair. While regulation of MMP activity has largely remained unclear and complicated, emerging evidence indicates a clear role for PKC signaling. Fleegal and colleagues have shown that PKC inhibition with chelerythrine chloride reduces BBB permeability, which lends credence to the idea that PKC may control expression and function of junctional proteins [25]. This is consistent with the suggestion that PKC may regulate other junctional proteins associated with the epithelial barrier [62].

The attempt to identify the role of specific isozymes involved in BBB permeability has produced multiple candidates depending on the model used, time points, and severity of injury utilized. In a global ischemia model, analysis of cortical microvessels demonstrated changes in PKC distribution, particularly that of PKC θ and PKC ζ , as well as phosphorylation activity of these isozymes. This suggests that these signaling molecules may further mediate tight-junction strength [63]. Others have suggested a role for PKC ζ in MMP regulation as well with Hussain and colleagues showing that PKC ζ activity is required for cytokine-induced activation of MMP-1, -3, and -9 [8].

Other isozymes identified as contributing to BBB regulation include PKC δ and PKC ϵ [62, 64, 65]. Notably, some proposed neuroprotective agents, such as doxycycline, have

reduced BBB breakdown and improved neurologic outcome while downregulating PKC δ , suggesting protective effects may be mediated via PKC isozyme modulation [66].

How PKC activation and translocation leads to MMP activation is not well elucidated but indications are that it may involve downstream signaling cascades mediated through the NF- κ B or MAP/ERK pathways [67, 68]. Work in other organs such as the kidney has shown other pathways and molecules may be involved in regulating MMP gene expression such as c-Fos and c-Jun, two cellular proto-oncogenes [68]. Other studies indicate potential modulation of cytokine-induced effects on MMP expression by PKC isozymes as well as identify a possible role of PKC-mediated phosphorylation of MMP-2 that then alters the activity of the protease [69-71].

In addition to the role the BBB serves in regulating solute passage and preventing vascular dysfunction, it also is responsible for maintaining the immunoprivileged state. PKC δ activation in endothelial cells studied *in vitro* revealed diminished neutrophil migration following stimulation with leukotriene B4 (LTB4) or tissue necrosis factor- α (TNF- α) without altering endothelial barrier integrity [72]. This would appear to be in contrast with previous findings in which PKC δ inhibition reduces endothelial dysfunction and improves BBB integrity [9, 59, 73].

Role in Recovery and Repair Following Neural Injury

Understanding the mechanisms associated with recovery and repair from neural injury remains the subject of intense investigation due to the potential promise for therapeutic development for a number of neurologic disease states ranging from ischemic stroke to TBI. Recovery/repair involves a complex array of simultaneously occurring processes including neural migration and angiogenesis. Recent work highlights roles for PKC-mediated signaling in each of these repair-associated components.

One of the greatest challenges in recovery/repair events is overcoming inhibitory activity attributed to the glial scar found at the lesion site. Much of the inhibition resulting from myelin and other molecules composing the glial scar is regulated by cPKC activity. By blocking PKC activity, neurite outgrowth has been stimulated both *in vitro* and *in vivo* despite the normally prohibitive presence of myelin [37]. This process likely requires a series of protease and metalloprotease-mediated steps, which may be regulated by PKC as discussed above. A prime example is that of semaphorin 3A (Sema3A), a guidance molecule that activates MMP-2 in a PKC α -dependent fashion [74]. Besides involvement in axonal guidance, PKC activation using bryostatin has been shown to increase mushroom spine number on neurons in a memory-specific manner [75].

Actual neurite outgrowth has been shown to be PKC ϵ -dependent in nature with an increase in PKC ϵ promoting nerve growth factor-induced growth whereas a decrease inhibits the process [10]. This overall pro-repair event appears consistent with work completed in the cardiovascular system in which sustained PKC ϵ inhibition following heart failure prolonged survival while reducing dysfunction through a variety of mechanisms, many of which are associated with the regulation of inflammatory pathways and inflammatory cell migration [14]. PKC δ , often thought of in a negative light with regards to impact on neural injury in the acute period, has been shown to modulate critical aspects of repair/regeneration such as

angiogenesis and cell cycle modulators, making PKC δ activation potentially beneficial in the chronic period post-injury. Specifically, Kim and colleagues demonstrated that PKC δ activation promotes NADPH oxidase activity and increases HIF-1 α levels, leading to angiogenesis [76]. PKC δ is also involved in cell cycle events based on actions on Cdk5 via p35 and consequently regulates cortical radial migration of neurons [77].

Effect of PKC Modulation on Learning and Memory

In addition to the role of PKC in neural migration and repair following injury, the effect of PKC on learning and memory has been well established in both healthy and injured animals. In *Hermisenda*, bryostatin was found to enhance memory acquisition at low-doses consistent with up-regulation of specific PKC isozymes but at high doses, bryostatin resulted in down-regulation and no recall of the associative training task [78]. One of the major cellular mechanisms associated with learning and memory, long-term potentiation (LTP), seems to require PKC-mediated signaling based on previous findings assessing the effect of phorbol esters and PKC inhibitors on LTP. While PKC has been described as necessary, but not sufficient, to induce LTP, it is clear that PKC isozymes such as PKC ϵ contribute to presynaptic control [10]. Furthermore, PKC modulators such as bryostatin have been shown to induce synthesis of proteins associated with memory consolidation, a fact that makes PKC modulators a potentially promising therapeutic target for conditions associated with memory impairment like Alzheimer's disease [79].

STUDYING PKC ISOFORM-SPECIFIC EVENTS: USING AVAILABLE TOOLS

Work in other organ systems, such as the cardiovascular system, has illustrated the importance of elucidating isozyme-specific events for the development of therapeutic agents. For example, Chen and colleagues showed that various isozymes may have similar effects with regard to one cellular function and opposing effects on another function, explaining contrasting findings often reported due to use of non-isozyme selective pharmacologic agents. Furthermore, the isozyme-dependence of cellular functions is of particular interest considering the elevation of multiple isozymes following injury and the idea that it is the balance struck between various isozymes that determine the overall outcome post-injury [33].

The elucidation of isozyme-specific events can be accomplished via genetic manipulations such as knockouts or through the use of pharmacologic inhibitors. Of the isozymes identified, at least four have been successfully mutated to produce null mice. This includes the γ , β , ϵ , and θ isozymes.

While null mice may provide insight into neurologic effects associated with a respective isozyme, animals are affected systemically as evidenced by β and θ -null mice, which have shown phenotypes consistent with immunologic changes [80]. One of the advantages of null mice though is the ability to target a given isozyme specifically, a significant challenge observed in studies using pharmacologic inhibitors that often inhibit one isoform class rather than a specific isozyme. For example, Go6976 has been described as inhibiting classical Ca²⁺-

dependent isozymes whereas Go6850 inhibits both conventional and novel isozymes [62, 74]. Other agents such as Ro 31-8220 have been shown to inhibit PKC α more selectively at low concentrations than high concentrations where additional PKC isozymes may be affected [75].

More recently, isozyme specific inhibitors have become available as well as the development of peptide-based specific inhibitors [1, 39, 57]. In addition to inhibitors of PKC, activators are also available and include promising therapeutic candidates, such as bryostatin, for a host of neurologic disorders [27, 39, 45].

FUTURE DIRECTIONS

Modulation of PKC using isozyme-specific pharmacologic and peptide-based inhibitors has yielded positive results in many preclinical studies, particularly in models of neurologic diseases such as stroke, TBI, and AD. Despite the apparent success of these studies and progression to early phase clinical trials in some cases, further studies are required to elucidate effects of common disease comorbidities such as aging, hypertension, and diabetes that have not been addressed in early preclinical studies. Similarly, many of the early preclinical studies have been conducted *in vitro* on neurons and have not addressed the potential of cell-specific or time-dependent effects. Furthermore, investigating signaling mechanisms associated with beneficial and detrimental PKC-mediated effects may lead to optimal therapeutic development by regulating multiple isozymes.

Alterations in PKC-Mediated Signaling Associated with Aging

The greatest risk factor for neurologic diseases such as ischemic stroke and AD is age. Most preclinical studies investigating PKC-mediated effects have not considered the role of age and the aging process. In one study that did address the effect of age on PKC in the brain demonstrated that cPKCs are substantially down-regulated in the cortex, striatum, and hippocampus of middle-aged animals compared to young animals. This down-regulation drastically changes the expression of substrates normally modulated by PKCs such as nitric oxide synthase (NOS) [61, 81]. Ultimately, such age-related alterations might predispose the brain to Alzheimer's disease, dementia, or other memory dysfunctions. In addition to PKC downregulation, the availability of the remaining PKC isoforms is disrupted. Most isoforms are not present in membranous regions due to a deficit in translocation. RACKs have decreased expression, up to 50%, in the aged brain. Without RACKs, PKCs are isolated to the cytosol of cells and cannot translocate even if activated. This leads to deficits in PKC-mediated neuronal plasticity and subsequent loss of LTP [13, 82]. To further complicate matters, oxidative stress and aluminum-induced neurotoxicity can cause PKC to transfer from the plasma membranes to the cytosol in aged brains [83]. In other tissues such as the heart, some RACKs may be functional while others are not. When an aged heart is confronted with increased preload, the expression of most PKCs is up-regulated. The RACKs specific for PKC β and PKC ϵ are still functional and can therefore initiate translocation. Since PKC β and PKC ϵ are overactive compared to the other regulatory cytosol-bound PKCs, the heart

hypertrophies and becomes fibrotic [36]. Considering the potential effect of age on RACK functionality, the ideal therapeutic would not depend on RACK function to mediate therapeutic effects on the targeted isozyme. Several drugs have been designed for such targets, and one specific drug of interest is Bryostatin-1. Bryostatin-1 is a potent activator of PKCs and also aids in increased translocation of PKC α [38, 78]. Kostyak and colleagues demonstrated that at baseline, PKC δ levels in the mitochondria of the heart were increased by approximately 40% in comparison to the normal adult heart. Similarly, aged hearts contained a nearly 2-fold increase in cytosolic PKC δ in comparison to adult heart. Perhaps most importantly, inhibition of PKC δ with KID1-1 reduced the increased infarct volume typically found in aged hearts to a level more consistent with the adult heart [57].

Elucidating Cell-Specific and Time-Dependent Events

Investigating the pathophysiology of neurologic diseases such as ischemic stroke, as well as developing therapeutics, is complicated by the numerous cell-types required for both normal homeostasis as well as the response to injury and subsequent repair/recovery. Being that each cell-type may respond differently to injury, or at least exhibit varying sensitivity to injury, may necessitate investigation of PKC-mediated processes independently for targeted therapeutic delivery. This idea is consistent with the varied findings across cell-types such as the role of PKC in modulating the inflammatory process and microglial adhesion, astrocytic control of MMP-9, and oxidative stress in neurons [52, 67, 72, 84]. Besides cell-specific events, previous work has shown time-dependent changes in PKC-mediated signaling in cerebrovascular studies. For example, PKC δ is activated early in cerebrovascular events and likely plays a greater role in the acute period whereas PKC α may play a larger role in the chronic period after injury. Therefore, studies more adequately addressing the timing of isozyme increases or decreases following an injurious event may lead to more appropriate therapeutic development [85].

Understanding PKC-Mediated Pathways

PKC-mediated signaling is involved in a wide array of homeostasis-associated pathways as well as those that occur in response to injury, but how precisely many cellular effects are controlled through PKC activity remains unclear. Numerous pathways have been implicated based on work in the brain as well as cardiovascular systems, but how these pathways all interact, and the role of various PKC isozymes in these pathways, remains to be seen. One signaling molecule of interest is glycogen synthase kinase 3 beta (GSK-3 β), an enzyme responsible for phosphorylating glycogen synthase kinase (GSK). Aging has been recognized as altering the ratio between phosphorylated GSK-3 β and GSK-3 β , a difference that coincides with worsened cardiovascular injury in aged subjects. Reperfusion results in a worsening of this ratio in the mitochondria of aged hearts, an effect prevented by PKC δ inhibition [57]. Vascular endothelial growth factor (VEGF) appears to exert its mitogenic and permeability-associated effects via PKC β demonstrating another potential pathway of investigation [86]. Lastly, the protein kinase Akt, which has been identified as regulating apoptotic events, has

been shown to correlate with PKC δ activity in the acute period after ischemia but not after reperfusion [58]. This indicates a potential link between the two pathways but also demonstrates the inherent complexity of signaling cascades, particularly those that overlap or display time-dependent effects.

CONCLUSION

PKC clearly plays a role in neurologic injury and represents a promising therapeutic target based on work in numerous preclinical models of AD, ischemic stroke, and TBI. Activity of some PKC isozymes has been demonstrated in both clinical samples and preclinical studies at a variety of time points post-injury, likely in an isozyme-specific fashion. While the precise mechanism through which PKC isozymes influences outcome following injury is unclear, evidence indicates a role for PKC in regulating apoptosis, blood-brain barrier integrity, inflammatory cell adhesion/migration, and multiple aspects of recovery/repair (Figure 2).

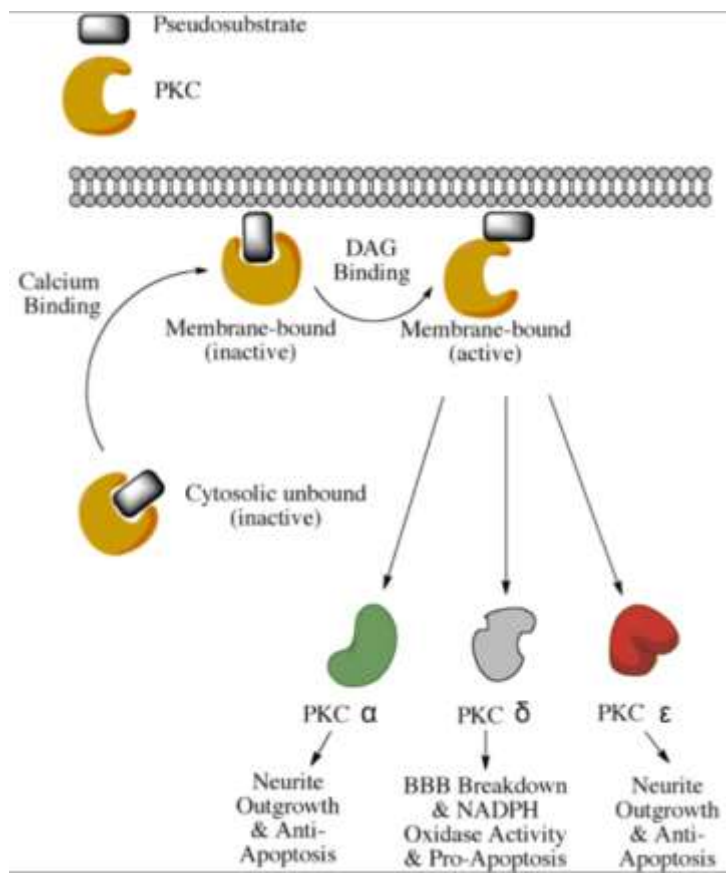


Figure 2. Schematic demonstrating generic PKC activation processes and subsequent isozymes with identified functions in neural injury/repair. Each isozyme likely has time-dependent and isozyme-specific actions that contribute to a range of cellular effects both during normal homeostasis as well as in response to injury.

Understanding isozyme-specific functions and how to most appropriately target these effects remains under investigation but recent improvements in peptide-based inhibitors provides promise in more clearly elucidating the role of a given isoform over time. As progress continues in this regard, successful therapeutics in preclinical studies that have progressed to clinical trials, such as Bryostatins-1, may potentially be used to carefully target individual isoforms and likewise increase functional recovery following a variety of neurologic injury types.

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Chapter 5

ROLE OF TRANSCRANIAL DOPPLER ULTRASONOGRAPHY IN CEREBROVASCULAR DISEASE

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ABSTRACT

Transcranial Doppler ultrasonography (TCD) is the only non-invasive diagnostic technique for obtaining reliable information about intracranial blood flow patterns in real-time. The information obtained from TCD often adds physiological component to the anatomical information obtained from other neuroimaging studies. TCD is relatively cheap, can be performed bedside for acute stroke patients as well as for patients with fixed vascular steno-occlusive disease. Some of the advanced applications of TCD include sonothrombolysis in acute ischemic stroke, monitoring for spontaneous emboli, right-to-left shunt detection and vasomotor reactivity, evaluation of vasospasm in patients with subarachnoid hemorrhage etc. These applications help in evaluating various stroke mechanisms, management plan as well as for prognostication purposes. TCD has been suggested as an essential ingredient of a comprehensive stroke center.

Keywords: Ischemic stroke, cerebrovascular disease, transcranial Doppler, cerebrovascular ultrasound

INTRODUCTION

Transcranial Doppler ultrasonography (TCD) is the diagnostic modality that provides the real-time information about various patterns of blood flow in major intracranial arteries in real-time. It was discovered for human use for measuring blood flow velocities in the middle

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cerebral artery (MCA) in patients with subarachnoid hemorrhage (SAH) [1]. Over past 3 decades, the clinical and research uses of TCD have extended immensely. In acute stroke patients, TCD can be used for a rapid bedside diagnosis of intracranial arterial occlusion as well as for monitoring the effect of various therapeutic approaches to achieve recanalization. Importantly, continuous monitoring of the intracranial occlusions in acute ischemic stroke patients during systemic thrombolysis is known to improve the chances of recanalization. The advanced applications of TCD such as right-to-left shunt detection, vasomotor reactivity testing, and emboli detection serve as important tools in the armamentarium of stroke neurologists for understanding the etiopathogenic mechanisms in acute stroke and individualizing the treatment strategies.

TCD has been demonstrated to be reliable, if performed by experienced sonographers [2, 3]. However, the results match favorably only when the TCD study is performed in close temporal relationship to various contrast angiographic studies [4, 5]. Furthermore, TCD is comparatively cheaper diagnostic technique that can be performed bedside and allows prolonged monitoring capabilities in selected cases.

FAST TRACK INSONATION PROTOCOL

In patients presenting with acute ischemic stroke ‘time is brain’. For patients considered eligible for intravenous thrombolysis, rapid performance of TCD is essential. Accordingly, a fast track insonation protocol was developed for such emergency situations [6].

‘Fast-track’ evaluation of the cerebral circulation requires an experienced sonographer. Clinical presentations in acute ischemic stroke often determine the insonation steps in this protocol. Therefore, for patients presenting with right-sided symptoms, the insonation begins with insonation of the right middle cerebral artery (MCA) (presumed to be normal and unaffected) side. This step helps the sonographer in assessing the quality of temporal acoustic window in addition to visualizing the normal spectral flow pattern and velocity profile of the index patient. This is followed by insonating the MCA on the affected side, starting at the mid-M1-MCA depth range of 50-58 mm. In patients with a normal MCA flow, distal MCA segments are evaluated followed by ICA bifurcation. Absence of MCA flow signals due to arterial occlusion can also be confirmed by deeper insonation across the midline from the contralateral window. Flow direction and pulsatility is determined in the ophthalmic artery (OA) via transorbital window on the affected side at depths of 50-58 mm followed by deeper insonation (60-64 mm) of ICA siphon. Basilar artery (BA) is insonated next to determine if compensatory flow patterns. Insonation of vertebral arteries (VA), OA, and ICA on the non-affected side, as well as posterior cerebral arteries, is performed whenever the sonographer finds time during the course of prolonged monitoring. The ‘fast-track’ protocol allows the sonographer to complete the evaluation of cerebral hemodynamics within few minutes and does not affect neurological examination, setting of intravenous access. This protocol does not contribute to any time delays in initiating the thrombolytic therapy in acute ischemic stroke.

The yield of fast-track TCD protocol can be further enhanced by combining it with cervical duplex sonography of the carotid and vertebral arteries and detect the lesions amenable to interventional treatment (LAIT) [7], defined as occlusion, near-occlusion, $\geq 50\%$

stenosis or thrombi in an artery supplying brain area. The sensitivity, specificity, positive and negative predictive values for TCD in diagnosing LAIT are 96%, 75%, 96% and 75% respectively. The corresponding values for carotid/ vertebral duplex were 94%, 90%, 94% and 90% respectively.

Catheter angiography in patients with acute ischemic stroke detects an arterial occlusion in 76% patients, when performed within 6 hours of symptom onset [8]. TCD performed by experienced sonographers has been found to match the findings of contrast angiography [2, 9]. TCD has a high sensitivity (>90%) for acute arterial obstructions located in the proximal MCA and ICAs. Up to 90% of patients who receive intravenous TPA within the 3 hours time-window demonstrate an acute occlusion on TCD, especially if the pre-treatment National Institute of Health Stroke Scale (NIHSS) is ≥ 10 points [110, 11]. However, the yield of TCD reduces significantly when performed later than 6 hours after stroke onset [6].

Unlike the smaller coronary arteries that move with each systolic beat, the intracranial arteries are more firmly fixed and can be easily insonated continuously with TCD. Therefore, in patients with an acute arterial occlusion, TCD can be used to monitor the time and patterns of arterial recanalization induced by systemic thrombolysis [11].

TCD has a sensitivity of 91%, and a specificity of 93% compared to angiography for MCA occlusion versus complete recanalization in patients receiving thrombolysis for ischemic stroke [12]. TCD has a moderate sensitivity (55-60%) for posterior circulation lesions [13]. Some of the limitations of TCD can be overcome by using the Power Motion Mode (PMD) TCD, invented by Mark Moehring and Merrill Spencer Figure 1 [14, 15].

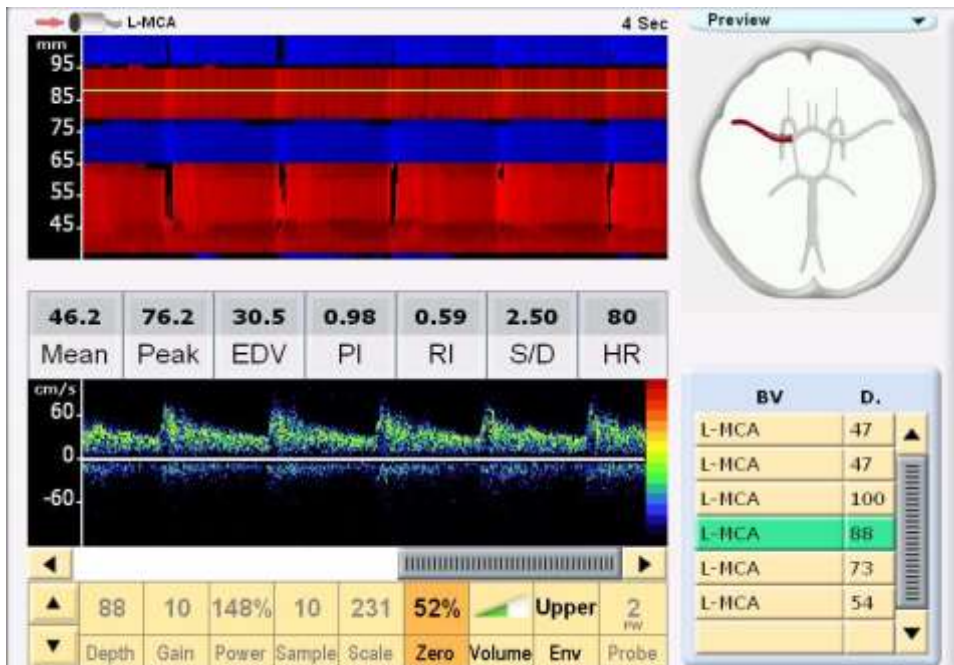


Figure 1. Normal transcranial Doppler using the temporal acoustic window with the screen appearance on SONARA TCD system. Upper frame represents the M-mode signatures from right middle cerebral artery, right A1 ACA and left A1 ACA. Lower frame shows the spectra obtained from a depth of 88mm, showing flow pattern in the contralateral A1 ACA.

TCD monitoring during acute stroke thrombolysis is often helpful in therapeutic decision-making for the next most efficient management steps, especially in some cases with persistent occlusion.

A persistent large vessel occlusion or stenosis in patients with acute and spontaneously resolving deficits heralds a greater possibility for the patient's deterioration within the next 24 hours [16]. Furthermore, TCD monitoring during the acute phase can help in guiding the blood pressure levels and head-positioning [17].

PROLONGED TCD MONITORING

No adverse effects have been documented in humans with the currently used ultrasound frequencies in the TCD systems. Prolonged TCD monitoring in acute stroke can help in early prognostication. Slow flow improvement and dampened flow signals are considered as less favorable prognostic signs. On the contrary, early recanalization of an occluded intracranial artery is often associated with rapid and dramatic recovery as well as good long-term outcome in majority of the patients [13]. Labiche et al. [18] showed that most patients who experience early clinical improvement within 2 hours of TPA bolus sustain this clinical benefit 3 months after stroke. On the other hand, Christou et al. [19] noted that among patients who had no change in the severity of neurological deficit or who worsened by 4 or more NIHSS points, none had complete recanalization within 300 minutes, implying that a persisting occlusion on TCD may represent severe ischemia.

Deterioration following improvement (DFI) is considered to be a clinical surrogate marker of intracranial arterial re-occlusion. Arterial re-occlusion following recanalization is believed to be the main underlying mechanism of this phenomenon [20]. Interestingly, patients with early reocclusion had better long-term outcomes than patients with no early recanalization on TCD. Similarly, Rubiera et al. [21] demonstrated a reocclusion rate of 12% in all TPA treated patients and among 17% of those who achieved recanalization. They found that at 3 months, patients with reocclusion and patient with persistent occlusion had significantly worse long-term outcomes than patients with persistent recanalization.

THERAPEUTIC TCD

Unlike the smaller coronary arteries that move with each systolic beat, the intracranial arteries are more firmly fixed and can be easily insonated continuously with TCD. Thrombolysis in Brain Ischemia (TIBI) flow grading system was developed to evaluate residual flow non-invasively and monitor thrombus dissolution in real time [22]. The TIBI system directs our attention to the relatively weak signals with abnormal flow waveforms that can be found along arterial stems filled with thrombi and elaborates on previous definitions of acute arterial occlusion. TIBI flow grades correlate with stroke severity and mortality as well as the likelihood of recanalization and clinical improvement.

Although intravenously-administered tissue plasminogen activator (IV-tPA) remains the only approved therapeutic agent in acute ischemic stroke, the rates of arterial recanalization are considerably low. del Zoppo et al. showed that only 26% of intracranial occlusions lyse

partially or completely after 1 hour of intravenous alteplase infusion [23]. In the PROACT II trial, only 4% of MCA clots showed complete recanalization when recombinant pro-urokinase was infused at the clot surface for 1 hour [24]. Accordingly, half of the patients remain moderately or severely disabled despite treatment with intravenous tPA [25].

Initial in-vitro experiments suggested that low mega-hertz (MHz) to kilo-hertz frequency ultrasound exposure substantially increased the thrombolytic effect of tPA [26-29]. Experiments using cadaver skull showed that 1 hour of 1-MHz TCD helped tPA to recanalize 90% of clots as compared to 30% rate when TCD exposure was limited to 30 minutes [26-28]. A small pressure gradient created by ultrasound waves is believed to provide an opportunity for more tPA molecules to bind with fibrin filaments and stream plasma along the clot, facilitating faster recanalization without harmful mechanical vibrations. Ultrasound enhancement of the thrombolytic effect of tPA does not appear to be mediated by thermal or cavitation effects since the mechanical index remains below 1. Various mechanisms responsible for ultrasound-enhanced thrombolysis include reversible disaggregation of uncross-linked fibrin fibers, microcavity formation in the shallow layers of thrombus, increasing uptake and penetration of tPA into clots as well as residual flow enhancement with microstreaming and vessel dilatation [27-29].

Operator dependency of TCD remains an important hindrance for TCD use as primary imaging modality at most of the centers around the world. Another important limitation, even encountered by experienced sonographers is the insufficient temporal acoustic bone window in 10-30% patients, depending on the ethnicity. Abnormal thickness and porosity of the temporal bone squama is believed to attenuate ultrasound transmission and is seen most commonly among African Americans, Asians and elderly female patients [30].

High rates of complete recanalization and dramatic clinical recovery were initially observed by Alexandrov et al. during tPA infusion and continuous monitoring with 2 MHz TCD [12]. In a small study on 40 stroke patients with mean NIHSS score of 19 points treated with IV- tPA were monitored with continuous 2-MHz TCD showed improved recanalization at 45±20 minutes after tPA bolus [12]. Recanalization was complete in 30% and partial in 40% of the patients while dramatic recovery during TPA infusion occurred in 20% of the patients, who had complete recanalization. Improvement by ≥ 10 NIHSS points or complete recovery was found in 30% of all patients at the end of tPA infusion and in 40% at 24 hours. This preliminary data led to a phase II randomized controlled trial called CLOTBUST, in which 126 acute ischemic stroke patients treated with IV-tPA were randomized to continuous TCD monitoring or placebo (63 patients in each arm) [31]. Complete recanalization or dramatic recovery within 2 hours was noted in 49% in the TCD group as compared to 30% in the control group ($p=0.03$). Only 4.8% patients developed symptomatic intracerebral hemorrhage thus showing a therapeutic benefit with no increased risk.

A multinational prospective observational study based on CLOTBUST trial protocol (CLOTBUST-PRO) is currently evaluating whether early recanalization is independently associated with better 3 month outcome in patients with intracranial arterial occlusions and correlates to a shorter time interval elapsed from symptom onset to IV-tPA bolus [32]. We have recently demonstrated the feasibility and efficacy of ultrasound-assisted thrombolysis in our local Asian patient population [33]. An example of successful recanalization of right mCA is shown in Figure 2.

Eggers et al. [34] evaluated the potential of transcranial color-coded sonography (TCCS)-guided, 2-MHz transcranial ultrasound in enhancing thrombolysis in patients with acute

occlusion of the M1 MCA and with contraindications to the thrombolytic therapy. Recanalization was observed more frequent in the ultrasound group as compared to the control group ($p=0.026$).

Subsequently performed TRUMBI trial [35] included 26 patients within 6 hour of symptom-onset. Patients receiving IV-tPA were randomized to 90 minutes of low-frequency (300kHz) ultrasound exposure or placebo. The study was stopped due to an increased incidence of intracranial hemorrhages (5 of 12 in TPA group versus 13 of 14 in TPA plus ultrasound group). The authors speculated that reverberations of the long wavelength ultrasound occurred inside the head, leading to 'hot-spots' in addition to the mechanical distortion of the microvessels causing parenchymal and even atypical hemorrhages to occur exclusively in the combined treatment group. However, the exact reasons for this observation remain unknown.

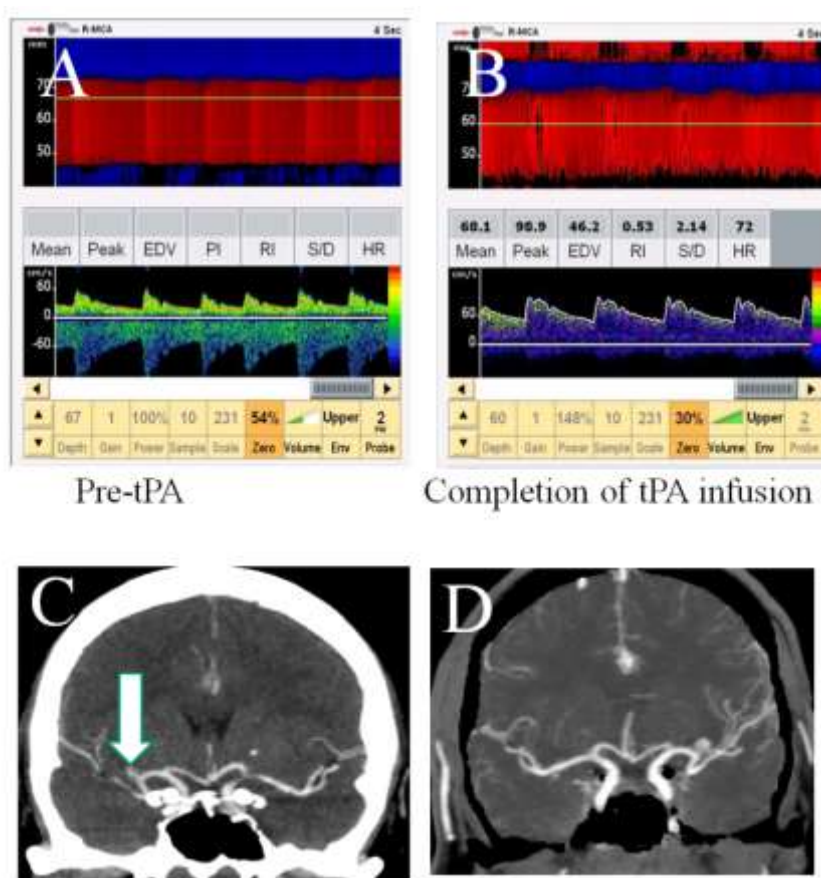


Figure 2. Neurovascular findings in a patient with acute right middle cerebral artery (MCA) ischemic stroke. Transcranial Doppler (TCD) performed at tissue plasminogen activator (tPA) bolus injections shows high- resistance TIBI 3 signals in right proximal MCA with flow diversion into the right ACA (A). The patient achieved complete recanalization in the form of normal flow spectra in right MCA (B) at the end of tPA infusion. This was accompanied by significant clinical improvement (NIHSS dropped from 11 to 2 points) during one hour. Brain CT angiography before tPA bolus had confirmed the occlusion of right MCA (C) that recanalized on the imaging performed on day 2 (D).

Ultrasound Contrast Agents and Sonothrombolysis

Although the diagnostic microbubbles or gaseous microspheres were originally developed to improve conventional ultrasound images, a new evolving therapeutic application for this technology is enhancing stroke thrombolysis [36]. When these microbubbles are exposed to an ultrasonic pressure wave, the gas expands, and the spheres oscillate, transmitting mechanical energy momentum to surrounding fluids and accelerating residual flow and possibly eroding at the thrombus [37, 38, 39].

First and second generation microspheres being air-filled, rise up in the container to make the infusion difficult [38, 39]. Their relatively larger size limits their free permeation through pulmonary circulation as well as the thrombus. On the other hand, the 3rd generation lipid coated microspheres are smaller in size and more stable in saline solution. They have a greater transpulmonary passage, producing higher concentrations within the arterial thrombus [38, 39]. In a recent study, it was observed that lipid microspheres permeated beyond the clot in 75% of subjects and improved the residual flow velocity in 83% of subjects [40].

A recently completed pilot trial reported higher recanalization rates with these 3rd generation Perflutren-lipid microspheres with no increase in symptomatic intracranial hemorrhage after systemic thrombolysis [41]. It was also demonstrated that PMD-TCD could quantify the dose of delivered microspheres and determine the minimum amount of microspheres needed to achieve constant flow enhancement and targeted drug delivery [42]. More recently reported TUCSON (transcranial ultrasound in clinical sonothrombolysis) trial evaluated 35 patients with 2 dose-tiers of Perflutren microspheres along with standard IV-tPA and continuous 2-MHz TCD monitoring. The trial concluded that Perflutren lipid microspheres can be safely combined with IV-tPA and ultrasound at a dose of 1.4ml. Although, higher rates intracranial bleeding were observed in the 2nd dose-tier, compared to standard intravenous IV-tPA, a trend toward higher early recanalization and clinical recovery rates therapy were noted [43, 44].

TCD IN CAROTID STENOSIS AND ENDARTERECTOMY

Due to their different acoustic properties compared to the circulating red blood cells, microembolic signals (MES), representing solid or gaseous particles within the blood flow, appear as transient signals of high intensity within the TCD spectrum [45]. They occur at random within the cardiac cycle and they can be acoustically identified by a characteristic ‘chirp’, ‘click’, or ‘whistle’ sound.

Identification of MES is especially important in carotid stenosis to identify patients who may benefit from early carotid endarterectomy (CEA) or more aggressive medical therapy. The CARESS trial revealed that the combination of clopidogrel and aspirin was associated with a marked reduction in MES, compared with aspirin alone [46]. In asymptomatic carotid stenosis, Spence et al. [47] demonstrated that TCD negative cases for MES will not benefit from carotid endarterectomy or stenting unless the operative risk is <1%. They even suggested that asymptomatic carotid stenosis should be managed medically, with delay of surgical intervention until the occurrence of symptoms or emboli.

Selection of patients for carotid revascularization is primarily determined by the presence of neurological symptoms and the severity of carotid stenosis on cervical duplex ultrasonography with confirmation provided by other imaging modalities [48, 49]. TCD can further assist in clinical decision-making by detecting hemodynamic effects of the carotid stenosis [50], identifying the tandem stenotic lesions [51] and determine exhausted vasomotor reactivity and select the high-risk group [52]. Monitoring for MES in patients with symptomatic and asymptomatic carotid stenosis is often used for risk-stratification [53, 54].

TCD is used at some centers for continuous monitoring of ipsilateral MCA flow as well as MES during carotid revascularization procedures. It can help in selecting patients for shunt during CEA and reduce peri-operative complications due to cerebral hypoperfusion [55] as well as for detection of cerebral hyperperfusion syndrome (if MCA velocities increase by more than 1.5 times pre-cross-clamp values and last more than 30 seconds after release of carotid cross-clamps).

TCD IN INTRACRANIAL STENOSIS

Intracranial arterial steno-occlusive lesions cause characteristic Doppler signals such as focal increases in velocity, local turbulence, a post-stenotic drop in velocity and various collateral flow patterns. Up to 10% of TIA and strokes can be attributable to intracranial atherosclerosis [56, 57]. Sensitivity, specificity, positive predictive value and negative predictive values of TCD are generally higher in anterior circulation than the vertebrobasilar circulation, owing to the more reliable anatomy of the former and technical problems in studying the latter [58]. However, PMD TCD has been recently reported with acceptable accuracy parameters, especially when TCD is performed early after the symptom-onset and by experienced sonographers [15]. There is an association between the degree of stenosis and recurrent stroke, with higher degrees of stenosis and the number of vessels affected having a worse prognosis [57, 58].

VASOMOTOR REACTIVITY

Cerebral autoregulation is the ability of brain to regulate its blood supply by compensating for changes in cerebral perfusion pressure. It enables constant cerebral blood flow over a wide range of systemic blood pressure by varying the diameter of the arteriolar sphincters [59]. The steno-occlusive lesions, their hemodynamic consequences and the alternative collateral pathways can be readily detected with digital subtraction cerebral angiography [60], computerized tomographic angiography (CTA) [61], magnetic resonance angiography (MRA) [62] or TCD [63].

Vasomotor reactivity (VMR) is the ability of the cerebral circulation to respond to vasomotor stimuli. This can be evaluated reliably with TCD by monitoring the real-time changes in the flow velocities in response to such stimuli.

The commonest agent to measure VMR is carbon dioxide (CO₂). Increased levels of CO₂ cause vasodilatation and increased cerebral blood flow. Whether the flow velocities represent cerebral blood flow volume (CBFV) remains a debatable issue. Sorteberg [63] stated that the

flow velocity can reflect CBFV and the velocity changes can be proportionate to the changes in CBFV if certain parameters are known and remain constant (e.g., angle of insonation, perfusion territory, arterial diameter) and the effect of only one stimulus is observed.

It is important to note that all these variables remain almost constant over the short period of time during VMR assessment by TCD. Monitoring of MCAs on both sides simultaneously further makes TCD more reliable since the magnitude of vasodilatory challenge as well as all other physiological factors are equal for the affected as well as the healthy MCA, facilitating identification and estimation of the vasodilatory reserve on the side of stenotic lesion.

Measuring VMR requires a proper setup with controlled conditions regarding the concentration of CO₂. Markus et al. [64] described a simple measurement of the MCA velocity in response to 30 seconds of breath holding and termed it as Breath-Holding Index (BHI). BHI is calculated as

$$\text{BHI} = \frac{\text{MFV}_{\text{end}} - \text{MFV}_{\text{baseline}}}{\text{MFV}_{\text{baseline}}} \times \frac{100}{\text{seconds of breath holding}}$$

where MFV is mean flow velocity.

A decreased VMR suggests failure of collateral flow to adapt to the stenosis progression. Therefore, impaired VMR measured by BHI can identify patients at higher risk of stroke who have asymptomatic carotid stenosis or a previously symptomatic carotid occlusion. Various studies, using different provocative measures for assessing the cerebral VMR, demonstrated a remarkable ipsilateral event rate of around 30% (30% risk of stroke/ TIA during about 2 years). In patients with persisting proximal arterial occlusions, hypercapnia can paradoxically decrease the residual flow velocity in the affected vessel at the expected time of normal brain vasodilation when blood pool is shifted to non-ischemic areas. This was described as “reversed Robin Hood” syndrome with the analogy to “rob the poor to feed the rich” in acute ischemic stroke patients [65]. This steal is detectable and measurable by TCD. Bilateral TCD monitoring can identify acute stroke patients with paradoxical velocity responses to hypercapnia or other vasoactive stimuli.

RIGHT-TO-LEFT SHUNT DETECTION

Patent foramen ovale (PFO) is common in general population with a prevalence of 10-35% in various echocardiography and autopsy studies. PFO with right-to-left shunts is considered a potential risk factor for stroke in patients with stroke of unknown etiology [66-74]. Trans-esophageal echocardiography (TEE) is considered as the gold standard for diagnosing PFO. However, TCD with agitated saline-air contrast mixture is a reliable method for detecting the presence of a right-to-left shunt. The sensitivity and specificity of contrast TCD has been reported to be 68-100% and 67-100% respectively [72]. Advantages of TCD

include calibrated valsalva maneuver and the ability to change body positioning during the test [69]. The TCD “bubble” test for right-to-left shunt is superior to transthoracic echocardiography, and possibly TEE. A recent study with TCD and TEE performed simultaneously, showed an almost perfect concordance in PFO detection and right-to-left quantification [70].

USE OF TCD IN SICKLE CELL DISEASE

Children with sickle cell disease have a significantly high risk of stroke before the age of 20 years [75]. Several studies have demonstrated that children aged 2- 16 years with this disease should be monitored with serial TCD evaluations for identifying the patients at an increased risk of stroke [76-77].

The Stroke Prevention in Sickle Cell Disease (STOP) trial [78] evaluated 130 children who had the time-averaged mean of the maximum (TAMM) velocities of > 200 cm/s in one or both of the MCAs or terminal ICAs at baseline TCD.

They were randomized to either blood transfusion or standard care. Over an average follow-up of about 20 months, there was one stroke in the 63 children randomized to transfusion and 11 strokes in the 67 children randomized to standard care, indicating greater than 90% relative risk reduction in stroke incidence in the treated population [79].

TCD FOR VASOSPASM STUDIES IN SUBARACHNOID HEMORRHAGE (SAH)

Diagnosis of vasospasm in patients with subarachnoid hemorrhage (SAH) was one of the first clinical applications of TCD [1, 80]. Vasospasm in SAH is a potentially preventable and reversible condition. Early recognition of vasospasm can be reliably detected with TCD and a timely appropriate therapeutic response can prevent or limit ischemic cerebral damage. The changes in velocity on TCD have been correlated with vessel narrowing measured by angiography, with MCA showing the best degree of correlation [81].

TCD is a useful tool in identifying vasospasm onset, its progression, and its severity as well as for monitoring the effect of therapeutic interventions [82].

TRANSCRANIAL COLOR-CODED DUPLEX (TCCD)

TCCD provides 2-dimensional gray-scale and color Doppler imaging of the circle of Willis. It enables the operator to obtain angle-corrected flow velocities from various intracranial arterial segments [83].

TCCD might be considered more accurate in the assessment of flow velocities than conventional TCD. However, no direct comparisons of both methods in pathologic conditions are available. The flow velocities in the intracranial arteries in healthy individuals are approximately 10% to 30% higher when measured using TCCD as compared to conventional

TCD. The difference is believed to widen in patients with intracranial stenosis. The comparison of the diagnostic efficiency of both methods at the same velocity thresholds cannot be valid and it is difficult to use the flow velocities defined by conventional TCD studies for analyzing intracranial stenosis by TCCD. For instance, WASID study [84] used a mean flow velocity threshold of 100cm/s for MCA. However, these thresholds were observed to be considerably different when TCCD was used. While Kimura et al. [85] found a peak systolic velocity of 180 cm/s as the optimal threshold, Baumgartner et al. [86] suggested peak systolic velocity thresholds of 220 cm/s for the detection of 50% stenosis of the MCA.

Although TCCD has been reported to have high sensitivity and specificity in the detection of a moderate intracranial stenosis [87, 88], it remains operator-dependent and the quality may be adversely affected in patients with insufficient temporal acoustic windows.

FUTURE DEVELOPMENTS

Although, TCD is a simple diagnostic ultrasound method from biophysics stand-point, it is the most complex test in vascular medicine. It offers a wealth of information at bedside about pathogenic mechanism of stroke. Converging lines of evidence suggest that TCD can help in identifying patients at high risk of subsequent cerebral ischemic events. It can be used to monitor the safety and efficacy of various therapeutic interventions rapidly and non-invasively. TCD detection of cerebral microemboli, especially during various therapeutic interventions and cardiovascular surgical procedures, is gradually establishing its role with a potential of being an integral component of the multidisciplinary therapeutic approach in patients with cerebrovascular diseases.

Ultrasound enhanced thrombolysis in acute ischemic stroke, especially with contrast microbubbles, has shown promising results. Advances in TCD continue to improve the capabilities of this technology. Transcranial ultrasound delivery in an operator-independent and dose-controlled manner being tested in a clinical trial, would further strengthen the existing armamentarium of stroke neurologists.

In summary, TCD is an invaluable tool in the diagnostic workup of stroke patients. TCD is also an evolving ultrasound modality with increasing diagnostic value and therapeutic potential. Future technological advances will overcome its limitations by easier bone window location and validated occlusion criteria to ease its use in acute stroke. Widespread availability of TCD would increase the use of this modality in acute stroke. Local validation of diagnostic criteria and test performance is required by vascular laboratory accreditation and quality assurance.

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Chapter 6

**INFLAMMATION IN ISCHEMIC STROKE:
MECHANISMS, DIAGNOSIS
AND MANAGEMENT**

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ABSTRACT

Inflammation plays an important role in ischemic stroke, not only in the development of atherosclerotic lesions, but also in the processes that lead to apoptosis and further neurological insults following stroke. Interestingly, inflammation also plays an important role in repair and recovery following stroke. This chapter will describe the pathophysiological basis of the inflammatory processes that lead to ischemic injury, the diagnostic imaging modalities (CT, MRI, SPECT, near-infrared fluorescence, etc.) available to predict inflammatory plaque rupture and subsequent thrombosis before it occurs, and the modulation of inflammatory mediators to prevent the spread of stroke and aid in recovery once the insult has developed. The role of the various inflammatory cytokines in cell death and proliferation after ischemia, the integrity of the blood-brain barrier, and the prevention and treatment of stroke will be mentioned. The challenges facing lifestyle modifications as well as prevention of ischemic stroke using current knowledge of the inflammatory processes involved in ischemia will be briefly discussed. Finally, the currently available as well as potential future treatments for ischemic stroke, rehabilitation and general neuroprotection will be discussed. Both conventional and alternative modalities of inflammatory modulation for neuroprotection and neurogenesis following ischemia will be described.

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I. INTRODUCTION

With an average of one victim every 40 seconds, almost 795,000 individuals experience a stroke every year. Thus, stroke is the fourth leading cause of death and the number one cause of disability in the adult population in the United States. Although the financial burden for stroke patients and clinical care is enormous, with an estimated cost of >70 billion dollars in 2010, effective stroke therapy has been very limited [1]. Hundreds of drugs that showed significant neuroprotection in animal models (peroxidase inhibitors [2], calcium channel blockers [3], NMDA receptor antagonists [4, 5] and many others) have failed clinical trials in humans [6] indicating that novel and more rigorous design and conduct of animal studies is required to choose the best candidate drugs for human clinical trials. This will increase the chances of successfully transitioning stroke medications. A growing body of scientific evidence from animal models of stroke as well as from human patients shows that inflammation and inflammatory modulators play a larger role in ischemic injury and recovery after stroke than previously credited. Classically, animal studies have shown that antagonizing various inflammatory components could decrease the area of brain infarction, suggesting that inflammation may have primary neurotoxic effects following stroke injury. However, more recent studies have shown that the role of inflammation in stroke is actually more complicated. There is an elaborate interplay involved in inflammatory modulation, resulting in an intricate balance between apoptosis and neuronal regeneration to achieve optimal outcomes following stroke injury. Cells of the neurovascular unit (neurons, astrocytes, pericytes and endothelial cells), microglia, and white blood cells recruited from the systemic circulation, as well as hundreds of cytokines, adhesion molecules and components of the coagulation pathway and the immune system are all involved in a tremendous cascade of events that regulate ischemic injury and contribute to repair and recovery after stroke. As examples of this interplay, this chapter will discuss the dual roles of cytokines, T cells, and microglia in stroke pathogenesis and recovery, including the use of inflammation as a tool in diagnostic imaging of stroke. The chapter will conclude with a discussion of the management options available for ischemic stroke prevention and treatment.

II. PATHOPHYSIOLOGY

Many molecular and cellular components are involved in inflammation as a response to ischemic injury in the brain parenchyma. Among the cells actively involved in inflammation within the brain are brain-specific cells such as microglia, astrocytes, and neurons, as well as circulating immune cells such as T cells and macrophages. Molecular contributors include adhesion molecules, cytokines, and chemokines. These molecules are regulated during the inflammatory process leading to the modulation of ischemic injury and cell death, notably apoptosis and necrosis. The permeability of the blood brain barrier (BBB) following stroke is also an important factor with regards to inflammation and recovery. For example, inflammation is one of several interdependent mechanisms that cause ischemia-induced vascular endothelial cell injury and disruption of the tight junctions of the BBB [7]. In addition, a recent study has suggested that lacunar strokes are associated with diffuse BBB dysfunction in the white matter and are not just ischemic in nature [8]. Cellular adhesion

molecules are important in maintaining the integrity of the BBB and for leukocyte trafficking. Matrix metalloproteases (MMP), a group of proteolytic enzymes heavily involved in tissue remodeling and degradation of extra-cellular proteins, are also involved in the inflammatory response. These proteolytic proteins disrupt the BBB through enzymatic degradation and remodeling of the extracellular matrix [9, 10]. After secretion by various activated cells including leukocytes, MMPs facilitate leukocyte migration and represent one group of enzymes involved in the inflammatory cascade [11].

a. Cytokines

Various cytokines regulate the inflammatory process: interleukin-1 beta (IL-1 β), interleukin-4 (IL-4), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-18 (IL-18) and tumor necrosis factor alpha (TNF- α), among several others. One key regulator of inflammation and cell survival is a downstream signal in the TNF- α pathway, the nuclear factor- κ B (NF- κ B).

NF- κ B is a heterodimeric protein composed of different combinations of members of the Rel family of transcription factors [12]. It is a ubiquitous transcription factor that regulates the expression of pro-inflammatory, pro-apoptotic, and anti-apoptotic genes [13-15]. Thus, NF- κ B acts as a double agent for neuronal survival and apoptosis. Likewise, NF- κ B signaling can have both an apoptotic as well as a neuroprotective effect following cerebral ischemia. For example, activation of NF- κ B in neurons could promote survivability, whereas its activation in glial or immune cells can lead to pathological inflammatory states [16]. Specifically, the p50 subunit is found in the adult brain, and transient middle cerebral artery (MCA) occlusion can induce the nuclear translocation and thus the activation of NF- κ B. Our recent investigation revealed age-dependent neural degenerative changes in p50 knockout (p50^{-/-}) mice. P50^{-/-} mice appeared normal at birth. At 6 and 10 months, the body weight of p50^{-/-} mice was significantly less than that of wild type mice and they started to die from age-related processes. In p50^{-/-} mice, morphological examinations showed: 1) aging and degenerative changes in the cortex and hippocampus including increased lipofuscin granules in neuronal cytoplasm, 2) abnormal capillaries, 3) dark and watery alterations and organelle accumulations, 4) apoptotic glial cells, and 5) DNA damage and caspase-3 positive neurons. These results suggest that p50 plays an important role in neurovascular remodeling, cell survival, and the aging process in the developing CNS [17]. In a consequent investigation in stroke mice, we showed that, after permanent MCA occlusion in p50^{-/-} mice, the ischemic infarct volume and cell death were significantly more severe than that in WT mice. Cell proliferation in regenerative regions such as the subventricular zone (SVZ) was hampered in p50^{-/-} mice. These results suggest that NF- κ B signaling is a neuroprotective mechanism [18]. This is consistent with other reports suggesting that with permanent occlusion of the MCA, decreased NF- κ B activity exacerbates ischemia and leads to increased neuronal death and brain damage [19, 20]. Conversely, some studies have suggested that using p50 null mice or inhibiting NF- κ B subunits in wild-type mice lead to decreased infarct size and increased neuroprotection in some areas of the brain. These data suggest that NF- κ B is a maladaptive factor and a target that should be blocked in transient ischemia [21]. NF- κ B activation may also lead to the proliferation of astrocytes, which regulate neuronal survival and regeneration

during ischemia and the post-injury period. In these ways, NF- κ B plays a dual role in cerebral ischemia, depending on the cell type in which it is expressed, the type and mechanism of ischemic injury (e.g., permanent vs. transient MCA occlusion), and the time point of investigation.

While the downstream mediator NF- κ B seems to be a main contributor to the duality of the inflammatory pathway, other cytokines such as TNF- α , IL-10 and IL-4 are also involved in cell signaling and the modulation of cellular responses involving microglia, T cells, and other inflammatory mediators. In human patients, TNF- α has been shown to be up-regulated in neurons, astrocytes and the invading immune cells at different time points after stroke onset, indicating that TNF- α may be involved in the acute and the delayed processes after ischemic stroke [22]. While it is interesting to see whether neutralizing TNF- α with the monoclonal antibody infliximab would benefit stroke patients, a randomized, double-blinded, placebo-controlled, pilot trial of infliximab did not show any improvement in patients with moderate-to-severe heart failure [23]. In another study to determine the levels of anti-inflammatory cytokines IL-10 and IL-4 in deteriorating ischemic stroke patients, it was shown that early worsening was associated with lower plasma levels of IL-10 in patients with sub-cortical but not cortical infarcts [22]. On the other hand, IL-18, a pro-inflammatory cytokine, increased within the first 24 hours after stroke onset and may be a predictive marker of stroke outcomes [24].

Our evolving understanding of how these cytokines function in the progression of the ischemic insult and how they interact in relation to the complex network of other molecules in the context of stroke is heavily dependent on studies in animal models and the cell types studied. The more knowledge we accumulate about the role of these chemical mediators, the more clinical trials we will be able to administer and the closer to stroke therapy we will be.

b. Microglia

Among the native brain cells involved in inflammation, microglia are highly implicated in mounting an inflammatory response. Microglia are glial cells that are believed to have a low turnover rate. They act as macrophages for the central nervous system, engulf infectious agents that penetrate the BBB and present antigens for T-cell activation. Resident microglia are believed to be bone-marrow/mesenchymal monocytes that reach the brain during development and differentiate into a resting or surveying phenotype within the parenchyma. In pathological conditions, microglia can proliferate substantially, become activated, and change their profile, becoming amoeboid in appearance with multiple branches and extensions [25]. IFN- γ can activate microglia which initiate a positive feedback loop, activating more microglia and creating a cytokine activation cascade including the release of TNF- α . Microglia also produce chemotactic molecules that can recruit T-cells to the site of injury [26].

Microglia are capable of producing large amounts of hydrogen peroxide and nitric oxide that can destroy both healthy and unhealthy cells. They also produce proteases, cytokines, glutamate, and aspartate, which can lead to direct cellular damage, axonal demyelination, and neuronal cytotoxicity. While their cytotoxic secretions are intended to destroy infected neurons and invading pathologic agents, the process may result in large amounts of reactive oxygen species that damage healthy neuronal bystanders. Thus, various anti-inflammatory

drugs such as indomethacin [27] and minocycline [28] have been used to target microglial activation and inflammation following acute stroke.

However, following the inflammatory response, microglia can serve to promote neural growth and rewiring through synaptic stripping, secretion of cytokines, and recruitment of neuronal progenitors to the infarct [29]. Thus, microglia exert both a supportive as well as an inhibitory role in inflammation and neural protection in the context of stroke. Increasing evidence implicates that microglia may have a supportive role and lead to beneficial steps in adult neurogenesis [25-31]. The effect of microglia on adult neurogenesis depends upon the subpopulation of microglia present in the brain, their state of activation and their functional phenotype. This activation state and phenotype is typically dependent on the chronicity of the injury. Following acute injury, neurogenesis occurs in the SVZ and the subgranular zone (SGZ) of the hippocampus. However, about 80% of these stroke-generated striatal neurons die in the first 2 weeks after they are formed [32]. Inflammation and microglial activation were initially blamed for the cytotoxic milieu that lead to the death of these newly formed neurons. However, neurogenesis can continue for over 1 year following stroke [33] and recent evidence has shown that the early cytotoxic, detrimental effects of microglia following stroke can later be transformed into a supportive state during the chronic, recovery phase. Microglia in these situations can support different steps in the process of neurogenesis; the survival, proliferation, and migration of neuroblasts and their differentiation to mature neurons [34]. This chronic, recovery phase must be distinguished from neuroinflammation, in which chronic microglial activation could lead to accumulated destruction in long-term pathological states such as that of Alzheimer's disease [35].

When activated microglia change into a chronic profile, their phenotype can either remain the same or change to another activation state, which can be either maladaptive or, potentially, neuroprotective. For example, microglia chronically exposed to LPS *in vitro* reduce the production of pro-inflammatory cytokines TNF- α , IL-1 α , IL-1 β , and IL-6 and free radicals such as nitric oxide, while retaining or enhancing suppressors of the pro-inflammatory function of microglia such as PGE₂ and IL-10 [31]. Secretion of PGE₂ inhibits microglial pro-inflammatory responses and down-regulates T-helper cells [36]. Despite chronic microglial activation, newly formed neurons that survive beyond the first month following deleterious microglial activation will continue to survive for at least 6 months following the insult [37]. Further, neuroblasts were rescued by microglia and microglia-conditioned medium *in vitro*, compared to neuroblast formation from SVC neural stem cells that were not cultured in conditioned media or co-cultured with microglia [38, 39].

Given the significant contribution of microglia to post-stroke injury and recovery, several imaging techniques have been developed to explore the dynamics of microglial transformation from the resting to the active states [40]. In summary, there is a delicate balance between the pro-inflammatory and anti-inflammatory effects of microglia on survival and neurogenesis, and much of this distinction results from the activation state and phenotype of microglial subtypes in the acute and chronic states following an initial injury to the brain. Microglia preserve homeostasis in the brain through secretion of extracellular signaling molecules and interaction with other cells. Thus, different activation states and phenotypes can result in microglial secretion of supportive as well as detrimental factors.

c. T Cells

Like microglia, different subsets of T lymphocytes play different roles in the modulation of the inflammatory response. Similarly, the activity of these T cells is up-regulated or down-regulated depending on the acuity of the injury. This activity can either be harmful to regenerating neurons through the acute release of pro-inflammatory cytokines and cytotoxins, or, later in the recovery phase, beneficial.

Initially following stroke, T cells enter the damaged region and contribute to tissue injury through non-specific mechanisms [41]. Studies have shown that suppressing this T cell entry can improve outcomes in experimentally induced stroke [42, 43]. Lymphocyte deficient mice have had consistently smaller infarct volumes and better functional recovery compared to wild-type controls in multiple studies involving ischemia and reperfusion [44-46]. Furthermore, mice lacking RANTES (CCL5), a chemokine attractant for T cells, have smaller infarct volumes following reperfusion as well [47]. However, this protection in T-cell deficient mice is lost when permanent ischemia without reperfusion is induced [48]. CD73, an ecto-5' nucleotidase expressed on a variety of cells including leukocytes, has been shown to rescue CD73 (-/-) mice from ischemic brain injury, and to decrease infarct volume and local accumulation of leukocyte subsets [49]. These studies about the damaging effects of acute inflammation have led to the development of various experimental therapies aiming at preventing T-cell infiltration into the brain and T cell activation following stroke, such as FTY720, FK506, Cyclosporin A, RTL551, and chemokine receptor antagonists [42, 50, 51].

However, the different subpopulations of T cells must be taken into consideration when approaching the treatment of stroke through T cell suppression. T lymphocytes are leukocytes derived from the bone marrow, then mature in the thymus and are essential in the immune system for clearing pathogens. T cells are subtyped based on their expression of co-receptor proteins (CD3, CD4, or CD8) and the types of cytokines they secrete. In this way, they can promote cell-mediated, inflammatory, humoral, and allergic responses. In the context of stroke injury, CD8⁺ cytotoxic T cells may contribute to post-stroke brain injury by extending the area of damage, while CD4⁺ regulatory T cells (Tregs) may limit these damaging effects. Natural killer T cells are necessary to prevent infections that might occur post-stroke [52]. Additionally, CD4⁺ helper T cells, especially Tregs, may contribute to repair processes and brain recovery in the subsequent days following stroke onset. Tregs limit the immune responses by releasing transforming growth factor beta (TGF- β) and IL-10, and in this way prevent the onset of autoimmune disorders [53, 54]. Tregs suppress inflammation and have also been reported to be neuroprotective, potentially by modulating harmful actions from microglia [55]. These cells may also function in stroke recovery through direct production of growth factors such as ciliary derived neurotrophic factor (CDNF) and TGF- β [56]. When T cells are depleted, neurogenesis is impaired implying that the nuclear factor of activated T cells (NFAT) is required for neuronal survival [57-59]. This action is primarily mediated through CD4⁺ CNS-specific T cells and Treg cells, partially through BDNF secretion, and appears to be lost in permanent ischemia models [60, 61]. Timing of post stroke T-cell targeted therapy is therefore an important consideration, so that the beneficial effects of T cell-mediated neuronal regeneration and anti-inflammatory actions are not unintentionally blocked.

On the first day following a stroke, the number of circulating T cells may be reduced, while on post-stroke days 7 and 21, the number of CD4⁺CD25⁺ T cells and Tregs is

increased [62]. In a subsequent study by Yan et al., there was an overall increase in Tregs in the peripheral blood following stroke for up to 3 weeks. Interestingly, the increase in Tregs occurred only in male stroke patients, and the decreased number of Tregs in females seemed to have an impaired ability to suppress T cell proliferation [63]. This may suggest an important role for Tregs in modulating the extent of stroke injury, especially that it has been noted that female ischemic stroke patients often have worse outcomes than males [64, 65]. Investigations into the use of Tregs to induce protection following stroke are still ongoing.

T lymphocytes may also have an important role in stroke-induced immunodeficiency syndrome. As early as 12 hours following ischemic stroke, a systemic immune depression can begin and last for several weeks. This suppression of the immune system which is mediated by hyperactivity of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, potentially through activation of the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) pathway, results in reduced numbers of T cells, atrophy of lymphatic organs, and infectious complications of the urinary and respiratory tracts within the first few days after stroke [66-70]. One treatment that has been used to suppress this response is propranolol [71]. Another mediator of stroke-induced immune depression is the vagal “cholinergic anti-inflammatory pathway”, in which the release of acetylcholine after vagal activation by pro-inflammatory cytokines results in the inhibition of further pro-inflammatory mediator release [72, 73]. Thus, in creating novel therapies to regulate T cell migration and activation, caution must be maintained to prevent further depressing an already weakened immune system.

Intercellular adhesion molecule 1 (ICAM-1 or CD54) is expressed on the surface of endothelial cells and leukocytes and aids in leukocyte adhesion and transmigration to brain parenchyma after stroke. Several studies have shown that ICAM-1 inhibition reduces neurological damage in animal models of stroke [74, 75]. Enlimomab, a monoclonal antibody against ICAM-1, has been tested in a clinical trial for stroke patients [76]. 625 patients were enrolled and Enlimomab was administered to 317 patients within 6 hours of stroke onset. Enlimomab was not found to be effective in the treatment of stroke and was even associated with more adverse effects primarily infections and fever. It was postulated that antibody production against Enlimomab together with a wide-spread immune system activation in stroke patients were responsible for the worsening of symptoms in the experimental arm [77]. In another test of anti-leukocyte adhesion strategy, a humanized anti-E/P-selectin antibody was found to be safe and effective in non-human primate stroke animals [78]. This antibody has not been used as a clinical treatment for human stroke patients yet.

III. DIAGNOSTIC IMAGING

Carotid atherosclerotic plaque rupture and thrombus formation are commonly known pathophysiological phenomena involved in the development of ischemic stroke. For this reason, recent attention has turned towards using diagnostic imaging to predict plaque rupture before it occurs. Plaques that are likely to rupture tend to have large, eccentric, necrotic cores and thin fibrous caps. Spotty calcifications and proliferation of the vasa vasorum, with heavy infiltration of inflammatory cells especially macrophages are typical characteristics of a mature plaque. Rupture mainly occurs in the shoulder where apoptotic cells and macrophages release proteolytic enzymes such as MMPs. Some imaging modalities have targeted the

identification of these metalloproteases for their predictive value. The identification of molecular surrogates to detect plaque rupture without diagnostic imaging is currently an ongoing area of research, but until these surrogates are found, noninvasive imaging modalities combined with clinical symptoms remain the mainstay for stroke prediction and treatment guidance [79].

a. Computed Tomography (CT)

CT has long been used to diagnose acute stroke when it occurs, particularly given the accessibility of CT to clinicians and its ability to rapidly image unstable patients in an emergency setting. Because of the availability and widespread use of CT for diagnosing stroke patients, studies have been performed to analyze the ability of CT as a predictive tool. For recurrent strokes following a minor stroke or transient ischemic attack, CT/CTA (CT-angiography) was not significantly different from MRI in its ability to predict stroke recurrence, indicating that CT/CTA could be an effective substitute for MRI at institutions where it is the imaging modality that is more readily available [80]. Recently, CT has been used in an attempt to predict plaque ulceration and rupture in at risk patients as well. In a series of 346 patients experiencing anterior circulation symptoms, multi-detector CT angiography (MD-CTA) was used to evaluate plaque presence, degree of stenosis, and ulceration in the carotid artery [81]. Measurements included plaque volume and composition: lipid rich necrotic core, fibrous tissue, and calcification. Plaque was identified in 185 of these symptomatic patients, with ulcerations in 38 of them, independent of the degree of stenosis. These results may help clinicians to identify rupture-prone plaques and improve risk stratification based on analysis of plaque composition and measurement of plaque volume, rather than simply based on a moderate-to-high degree of stenosis.

b. Magnetic Resonance Imaging (MRI)

MRI has proven to be a useful diagnostic tool in distinguishing plaque components using non-invasive imaging. MRI can differentiate fibrous cap, necrotic cores, intraplaque hemorrhage and calcification, and thus can correlate with clinical symptomatology [82]. Studies on local hemodynamic forces and shear stresses near the area of carotid arteriosclerosis have been performed to allow for noninvasive dynamic assessment of developing atherosclerotic plaques [83].

Other studies have focused on the development of targeted molecular contrast agents such as P947, an MMP inhibitor that has been coupled to gadolinium chelate [84]. MMP expression has resulted in higher signal intensities in the shoulder regions and fibrous cap in MRI, as expected [85].

Disadvantages include poor reproducibility and difficulty in imaging small arteries, as well as unknown probe toxicity. However, high-resolution MRI as an imaging tool with sub-millimeter spatial resolution and lack of harmful radiation could prove to be a very useful tool for detecting MMP activity, evaluating atherosclerosis, and potentially predicting the likelihood of plaque rupture and guiding treatment [86].

Alternatively, USPIO-MRI (using ultra-small super-paramagnetic iron oxide –USPIO- as a cell specific contrast agent) has been successfully applied for the *in vivo* imaging of macrophages as markers of inflammation in stroke patients. USPIO-MRI allowed the imaging of macrophage infiltration into the ischemic brain [87, 88] and the identification of inflamed carotid plaque lesions in stroke-prone patients [89].

c. Ultrasound

Ultrasound modalities may be invasive or non-invasive. Intravascular, invasive ultrasound produces high-resolution images that have been found useful in distinguishing the morphological characteristics of carotid plaques and predicting stroke occurrence. The safety of intravascular ultrasound with and without an embolic-protection device was evaluated in a series of patients with >50% internal carotid artery stenosis and found to be safe [90]. However, invasive methods of ultrasound are not always necessary.

Plaque mobility is a salient feature in non-invasive ultrasound imaging for predicting the likelihood of plaque rupture. Progressive ischemic symptoms are more frequently associated with mobile plaques, and histology shows that mobile plaques tend to have a higher incidence of mural thrombus and a higher ratio of necrotic core to plaque area [91]. The mobility of the plaque has been described as the “jellyfish sign” on B-mode ultrasound, because the plaque surface moves in a manner that is discordant with the arterial pulse. The jellyfish sign suggests potential rupture of the fibrous cap and thrombogenic factor release into the arterial lumen. It is associated with a repeated stroke shortly following diagnosis and has been found to be more predictive of high-risk plaque vulnerability than intra-plaque hemorrhage [92].

d. Single Photon Emission Computed Tomography (SPECT)

Use of SPECT for monitoring patients at risk of atherosclerosis is primarily limited by the amount of radiation delivered with radiotracers necessary for imaging. However, it does have the benefit of being non-invasive, sensitive, already in clinical use, and more available than PET. Most studies to date involving the identification of high-risk plaques have been performed in animals, using MMPs as a high-risk marker of plaque vulnerability, given its role in the destabilization of atherosclerotic plaques. The first studies involving SPECT to identify inflammatory components in atherosclerotic plaques used New Zealand white rabbits with balloon-induced endothelial injury followed by a high-fat and cholesterol diet [93, 94]. MMP inhibitor uptake was easily visualized by microSPECT, following intravenous administration of the tracer. The results were confirmed with immunohistochemistry, and the uptake of the MMP inhibitor was decreased following diet modification and statin therapy. Subsequent studies showed similar results with different MMP inhibitors, radio-probes, and radiotracers [95]. DTPA-SPECT has been used in the period after acute stroke to evaluate the integrity of the BBB and its correlation with post-stroke seizures and worsened post-stroke outcomes as well [95, 96].

e. Near-Infrared Fluorescence (NIRF)

NIRF has the benefit of being a non-invasive, non-irradiating form of diagnostic imaging. It operates in the near-infrared spectrum, creating fluorescent images that can detect MMP activity. Because of the way in which the protease cleaves the fluorochromes, and the depth of penetration, there is an amplification of fluorescence and a relative improvement in the image as compared to SPECT for *in vivo* imaging [97]. NIRF has been used to examine MMP activity following cerebral ischemia as well [98]. Not only has NIRF been used to identify MMP activity, it has also been used to examine fibrin deposition and progression to thrombo-embolic stroke in mice, using activated Factor XIII (FXIIIa) as a target [99]. Despite these promising results, limitations of NIRF include low sensitivity and limited spatial resolution, unknown dye toxicity profiles, and studies have been performed only in animals and *ex vivo* human artery plaques [86].

NIRF was first applied to human carotid artery plaques in 2009 [100]. This study allowed for the detection of differential topographic NIRF signals in an excised plaque, with low-intensity cold spots and high intensity hot spots. However, anatomical topography and NIRF probe detection do not completely correlate, indicating that NIRF may be detecting more molecular susceptibility that is not always identifiable with conventional imaging. This ability to potentially identify more vulnerable plaques could therefore improve selection criteria for surgical interventions, if applied to human patients *in vivo*.

IV. PREVENTION AND TREATMENT

a. Prevention

Although there are some who disagree, conventional wisdom and current guidelines suggest that eating a diet high in fresh fruits, vegetables, and low-fat dairy products, combined with a high intake of dietary and soluble fibers, whole grains and protein from plant sources and decreasing consumption of saturated fat, cholesterol and sodium, can aid in the prevention of stroke [101]. Other lifestyle modifications that have shown strong evidence for stroke prevention include participation in moderate exercise (30 to 60 minutes) 4 to 7 days per week. Maintaining a low-normal body mass index, smoking cessation, and decreasing alcohol consumption are also important factors in reducing stroke and cardiovascular risks, particularly since smokers are at almost 4 times increased risk for stroke relative to nonsmokers.

Guidelines for secondary stroke prevention include intravenous tissue-type plasminogen activator (tPA), anti-hypertensives, anti-thrombotics, anticoagulants for atrial fibrillation, lipid-lowering medications, smoking cessation, and weight loss education. There are, however, unfortunate regional variations in care, with many individuals still receiving suboptimal stroke therapy [102]. Management of hypertension, cholesterol, diabetes, and atrial fibrillation should be priorities for stroke prevention. In terms of blood pressure management, diastolic blood pressure should be maintained at <90 mmHg, and systolic blood pressure should be <140 mmHg. Blood pressure targets should be lower in those with a history of stroke or diabetes. Lipid management with statins should be initiated in those at

risk of stroke, since a 20 to 30 percent relative risk reduction in recurrent vascular events has been reported for patients without coronary artery disease and who have a history of stroke after lipid management with statins [101].

b. Treatment

b1. Currently Available Therapies

Stroke is one of the leading causes of morbidity and mortality worldwide [1] and yet there is a paucity of acceptable treatments that improve neurological recovery. The current guidelines include emergent management and supportive care for stroke patients, along with early thrombolysis, volume expansion for hemodilution, pressure support or anti-hypertensives as indicated [103]. Mechanical Embolus Removal in Cerebral Ischemia (MERCI) devices for extraction of intra-arterial thrombi may also be used in selected patients within 6 hours of MCA occlusion, but further trials on intra-arterial thrombolysis therapies are needed, despite current recommendations from the European and American Stroke Associations. Recommendations from the American Stroke Association (ASA) further include initiating anti-platelet therapy with aspirin within 24-48 hours of ischemic stroke, assessment of swallowing with appropriate nutritional support if needed, early ambulation and deep venous thrombosis prophylaxis, and treatment of recurrent post-stroke seizures as well as initiation of antibiotic therapy for suspected infections. Additional treatment of acute neurological complications include measures such as mannitol to lessen the risk of edema, hyperventilation to decrease intracranial pressure, ventricular drain placement for acute hydrocephalus, and decompressive surgical evacuation of a space-occupying cerebellar infarction [103].

Aside from supportive care, currently available treatment modalities for acute ischemic stroke primarily target tissue reperfusion through thrombolytics such as tPA in the immediate period following injury. The use of tPA is, however, limited by time, having a small window of administration of approximately 3 - 4.5 hours following stroke, and carries with it the risk of hemorrhage, since it works by clot dissolution. Newer generation thrombolytic agents have shorter durations of action and are more fibrin-specific, but of the recombinant tPAs available, only alteplase has been FDA approved [103].

After injury has occurred, the most promising method of neurological recovery remains prevention of further stroke or stroke-related complications (vasospasm, infection, hemorrhage, reperfusion injury) and rehabilitation. Peripheral stimulation and physical therapy can improve functional recovery and neurovascular plasticity in the central nervous system. This has been confirmed by animal studies using peripheral stimulation following focal ischemia in mice. Peripheral stimulation resulted in increased angiogenic factors such as VEGF, restoration of local cerebral blood flow, enhanced neuroblast migration through up-regulation of chemokines such as those in the SDF-1/CXCR4 signaling pathway, and enhanced neurogenesis [104].

Another available therapy to limit brain injury and promote neurological recovery following stroke involves mild to moderate hypothermia (reducing body/brain temperature by 3-5°C). Both animal and human data show that hypothermia is protective against various brain insults [105-109]. Clinical evidence shows that a 3 to 5°C reduction is generally safe

and effective in reducing cell death and improving outcomes after brain ischemia [110]. Moderate hypothermia reduces intracranial pressure, increases cerebral perfusion pressure [111], and reduces the risk of brain edema and hemorrhage [112]. In the clinic, hypothermia is the only neuroprotective therapeutic treatment for cerebral ischemia that has successfully managed the transfer from bench to bedside, and it is an approved therapy for patients after cardiac arrest and children with hypoxic-ischemic encephalopathy [113].

Hypothermia protects the brain in multiple ways [114]. It reduces cerebral demand on neuronal activity and prevents excitotoxicity [109]. In addition, it suppresses the production of oxygen free radicals and inhibits pathways related to BBB degeneration [114]. Hypothermia-induced neuroprotection is also associated with blocking many of the processes leading to apoptosis [115] and suppressing the inflammatory response observed in brain ischemia [116-118]. Lending further support for the beneficial effects of hypothermia therapy, there is a general consensus that pyrexia and temperatures $>37^{\circ}\text{C}$ following ischemia are associated with poor patient outcomes [119]. Higher body temperature (hyperthermia) of approximately $37\text{--}40^{\circ}\text{C}$ after circulatory arrest is a common clinical symptom and it worsens outcomes [119]. There is consistent evidence that early (noninfectious) hyperthermia after stroke is associated with a marked increase in morbidity, mortality, and size of the brain infarct [119]. After a severe MCA thrombotic infarct, brain temperature exceeds body core temperature by up to 1.5°C [109] and pyrexia is independently and significantly associated with indices of stroke severity and 90 day mortality [120]. Post-stroke pyrexia is associated with significantly higher morbidity and mortality rates (43% increased risk of fatal outcome) as compared with afebrile patients [121]. In a large hospital-based observational study of patients with acute ischemic stroke, body temperature above 37.5°C was associated with poorer outcome compared to normothermic patients [122]. Supporting the importance of controlling post-stroke temperature, decreasing body temperature from 36.8 to 35.5°C resulted in $\sim 50\%$ risk reduction in fatality [123].

On the other hand, it is generally agreed that severe hypothermia, i.e., reducing body temperature below 30°C , may cause adverse effects such as increased risk of hemorrhage, cardiac, pulmonary or renal damage, electrolyte imbalance, and infections. Hypothermia can cause decreases in systemic blood pressure, especially during the cooling phase [124]. There are only few circumstances where intensive cooling can be applied in clinical settings. For example, in major vascular and neurological surgeries, the target of cooling is sometimes EEG silencing, an indication that cerebral metabolism has slowed to near-non-existence [125]. However, such extensive cooling would be impractical for awake patients, and is only applicable primarily to the anesthetized patient undergoing surgery. In general, for every 1°C decrease in temperature, there is a proportional 6% drop in cerebral metabolic rate [126]. Cooling also leads to a decrease in inflammatory mediator release by altering IL-6 and IL- 1α resulting in decreased cell death and apoptosis in animal models [116]. Cooling can be induced via endovascular as well as external modalities, such as indwelling catheters and cooling blankets. Immediate initiation of hypothermia should, theoretically, protect injured tissues and cells from further damage resulting from inflammation, edema, excitotoxicity, and increased metabolic turnover. Rewarming must then be performed much more slowly, as rapid rewarming can lead to an increase in brain edema and cerebrovascular volume due to the rapid increase in cerebral blood flow that comes with decreased cerebrovascular resistance [127].

One recent meta-analysis showed that mild hypothermia to 33°C does not significantly affect stroke severity and has no impact on mortality [128]. However, therapeutic hypothermia continues to be studied in an attempt to elucidate the ideal degree of cooling and the settings in which it can be used, with the promise of randomized controlled trials underway [128, 129]. Those studies that do show a benefit of cooling for ischemic stroke indicate that cooling must be initiated immediately following ischemia, so that target temperatures are reached in the tissues of interest rapidly following injury. Another study of the acute effects of localized cortical hypothermia on dendritic structural damage following ischemia showed that neither moderate hypothermia to 31°C nor deep hypothermia to 22°C blocked the onset of dendritic blebbing or dendritic spine loss in mice. However, during reperfusion, hypothermia did more consistently promote recovery of dendritic structure and spines [130].

Mild hypothermia to 34°C has been shown to decrease infarct volume and reduce brain swelling in experimental stroke when combined with thrombolysis [131]. In the same study, rats exposed to mild hypothermia and tPA had improved neurological scores relative to rats exposed to tPA alone, indicating that hypothermia can reduce the tPA-related side effects of reperfusion. Even though hypothermia could be potentially beneficial and neuroprotective in ischemic stroke, physical cooling is limited by time and increased whole body energy consumption. Other side effects of severe hypothermia include hypoglycemia and alkalosis [132, 133]. Not only does surface cooling require 3-6 hours to achieve a 2-5°C temperature reduction, but shivering and sympathetic activation are both homeostatic mechanisms that could increase metabolism and oxygen consumption, potentially contributing to worsening ischemia. Therefore, physical hypothermia is often limited to sedated or non-responsive patients in an attempt to prevent shivering.

b2. Novel Therapies

Since the available treatments for stroke are primarily preventive and supportive, and those that are meant for the acute treatment of stroke (tPA and cooling) come with undesirable side effects, ongoing research is developing new modalities of neurological preservation, recovery, and regeneration.

Pharmacologically Induced Hypothermia

Because of the complications associated with physical cooling, research has developed drugs to induce hypothermia pharmacologically. Paracetamol can induce hypothermia resulting in only a ~ 1°C decrease in core temperature and, thus, is insufficient for neuroprotection.

Newer pharmacological agents being used in experimental trials are meant to decrease the “set-point” in the brain’s thermoregulatory center, to more safely produce clinically significant hypothermia without altering other physiologic parameters.

Targets for pharmacologically induced hypothermia include transient receptor potential vanilloid-1 (TRPV1) agonists and neurotensin receptor 1 (NTR1) agonists [134-136]. TRPV1 receptor agonist rinvanil at 25 mg/kg induces hypothermia to about 33.4°C 30 minutes after injection in animals, returning to normal temperatures about 2.2 hours later. Higher doses resulted in a similar degree of hypothermia, but for longer time periods. Rinvanil could be given intracerebroventricularly and it reduces both O₂ consumption and skin temperature [137].

In an effort to overcome the limitations by forced physical cooling, we have generated novel neurotensin compounds that are centrally acting on the "thermoreceptor" neurotensin receptor 1 (NTR1) and could effectively induce "regulated hypothermia" only minutes after administration without triggering shivering. Post-ischemic administration of these compounds attenuates ischemia-induced brain injury and promotes functional recovery in experimental stroke models of rodents [138]. These compounds thus provide a novel means of hypothermia therapy and further investigations on the safety and effectiveness of these hypothermic drugs may help to translate this pharmacologically induced hypothermia therapy to human patients.

Erythropoietin

A crucial component to ischemic stroke therapy is restoration of blood supply. Thrombolytics can be used immediately in an attempt to dissolve clots and reduce ischemic time, but these agents carry with them the risk of hemorrhage, resulting in potentially worsened neurological damage and increased infarct size. However, newer agents such as Erythropoietin are currently being studied in an attempt to restore local blood supply and achieve neuroprotection without paradoxical reperfusion injury.

Erythropoietin is a hematopoietic cytokine that stimulates erythropoiesis and has been observed to have neuroprotective qualities in the central nervous system [139, 140]. It is expressed on different cells within the central nervous system: neurons, astrocytes, and endothelial cells [141]. Erythropoietin has an anti-apoptotic effect on neurons and other cell types, including cerebral vascular cells, and its neuroprotective effect is dependent on protein kinase B activation and mitochondrial maintenance, as well as regulation of caspase activation [142-145]. Erythropoietin has also been shown to have anti-inflammatory properties, including blocking a secondary rise in IL-1 β and other inflammatory mediators [146]. When administered 30 minutes prior to ischemia and once per day after an ischemic event in animal studies, recombinant human Erythropoietin treatment was found to attenuate ischemia-induced cell death, decrease infarct size, enhance post-ischemia angiogenesis, and restore local cerebral blood flow to the post-ischemic cortex [147]. Erythropoietin has also been shown to reduce endothelial cell death in the ischemic penumbra in neonatal rats [148].

In clinical trials, treatment with *Darbepoetin alfa* for prophylactic neuroprotection prior to aortic surgery seemed to have some benefit [149], but this study was halted prematurely when a multi-center German study showed possible harm from using Erythropoietin in the treatment of acute ischemic stroke [150].

Due to concerns regarding thrombo-embolic complications from Erythropoietin, most of the German Multicenter EPO Stroke Trial patients received systemic thrombolysis with tPA, when eligible. Unfortunately, patients who were administered tPA and Erythropoietin had a higher overall mortality than those administered tPA alone, suggesting that there may be yet unknown harmful interactions involved with administering Erythropoietin in combination with tPA for the treatment of acute ischemic stroke. However, in a previous study by the same group, there was an overall benefit with decreased infarct size and improved functional outcome when high-dose intravenous Erythropoietin was administered to a small group of patients <80 years old, as compared to placebo [151]. One recent study involved the use of pegylated liposomes for the targeted drug delivery of Erythropoietin to damaged areas of the brain to decrease cerebral ischemia/reperfusion injury [152]. Another group used hydrogel for direct, concentrated delivery of Erythropoietin to stimulate neurological recovery following stroke in animal models [153]. These new, targeted delivery methods may result in improved

avoidance of side effects and safer method of Erythropoietin administration, but this has yet to be confirmed in human studies.

Other Neuroprotective Agents

Agents such as valproate, DL-3-n-Butylphthalide and the neural peptide apelin have been shown to be neuroprotective in various studies, and may eventually have clinical benefit when applied to stroke management. Valproate is an anti-convulsant that has been shown to be neuroprotective following transient global ischemia in rats [154]. It significantly increased the density of neurons in the hippocampal CA1 region one week after the ischemic event, and suppressed microglial activation and number of microglia, indicating a role for valproate in inhibiting the cerebral inflammatory cascade as well. Apelin, an endogenous neural peptide, has been shown to be neuroprotective in cortical cultures, reducing markers of neuronal apoptosis [155]. DL-3-n-Butylphthalide is a cytoprotective compound synthesized from the seeds of *Apium graveolens* Linn (Apiaceae), commonly known as celery, and it has been shown to prevent neuronal death after focal cerebral ischemia in mice through the JNK pathway [156].

Other putative neuroprotective agents include thrombin, honokiol, curcumin and their derivatives [157-159]. Whether these compounds are effective in clinical trials for neuroprotection and recovery following ischemic stroke is yet to be determined. Electroacupuncture could also provide a promising field of research for stroke rehabilitation and neuroprotection as well, and has been shown to attenuate cerebral ischemic injury through an anti-inflammatory action in animal models [160]. These results need to be more consistently reproduced before the use of such therapies gains wide acceptance in the medical community.

Cell-Based Therapies

Successive failures in translating stroke drugs to the bedside prompted scientists to look for therapy in stem cells. Stem cell transplantation is a promising therapy for ischemic stroke to replace lost tissue and provide trophic support that limits inflammation and enhances endogenous repair mechanisms [161]. Regeneration of the brain after ischemia requires an active replacement of blood vessels, neurons and glial cells and the integration of the endogenously regenerated or transplanted cells in functional networks. Studies from our lab and others have shown that transplanted stem cells survive, differentiate, enhance functional recovery [162-164] and suppress inflammation [165, 166] after ischemia. Stem cells differentiate to neurons, glial and endothelial cells and provide trophic support that enhances endogenous repair mechanisms (angiogenesis and neurogenesis) [167]. Many types of stem cells have been used as therapy for stroke: embryonic [168], bone marrow [169, 170] and cord blood [171] cells. Studies have also shown that transplantation of human embryonic stem cells improves functional recovery after ischemic stroke [172, 173]. Recently, scientists, using a cocktail of transcription factors, reprogrammed mouse [174] and human [175] fibroblasts to pluripotent stem cells which are able to differentiate to all the three germ layers. Moreover, fibroblasts have been directly converted to neurons and neural progenitors [176-178]. The potential of these induced cells in the treatment of ischemic stroke is being investigated in animal models of stroke. There are tens of clinical trials that are currently testing the potential of hematopoietic bone marrow mesenchymal or adult neural stem cells for the treatment of ischemic stroke. A full list of these trials can be found on

clinicaltrials.gov. Most studies are either recruiting or in progress and no large randomized trials have been performed [179]. Geron, a biotechnology company and the first to start a clinical trial for human embryonic stem cells in spinal cord injury patients, has halted its trial due to money scarcity. Whether stem cells will be effective in the treatment of human stroke patients is yet to be determined.

SUMMARY AND CONCLUSION

Cytokines, microglia and cells of the immune system interact in a yet to be disclosed fashion to produce a complicated inflammatory cascade of events that contribute to tissue injury and regeneration. More studies are required to fully understand how these cells and molecules interact before therapies are to be developed. Current guidelines recommend quick diagnosis and treatment of ischemic stroke with CT/MRI guided imaging, supportive therapy, and immediate thrombolytic treatment. This is followed by prevention of stroke recurrence through dietary and lifestyle modifications, as well as anti-platelet therapy and management of co-morbidities. There are currently no agents approved for usage to increase neuronal regeneration, but research is underway. Many of these novel agents target inflammation and apoptosis in an effort to decrease neuronal destruction and infarct volume following ischemia-reperfusion injury. The complete inhibition of apoptosis and the inflammatory cascade would clearly be maladaptive for a number of reasons, as both are necessary processes to promote accurate neuronal mapping and to protect the host from infections and other onslaughts. However, the overwhelming surge of cytokines and cellular death that immediately follows brain injury can be detrimental as well. As with all things, a harmonious balance must be struck so that the endogenous inflammatory mechanisms that create collateral damage are coaxed into the ideal state to protect and promote neuronal regrowth and functional recovery.

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Chapter 7

ISCHEMIC STROKE SUSCEPTIBILITY GENE RESEARCH: LESSONS WE LEARNED

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ABSTRACT

In molecular-genetic research of stroke, candidate gene association studies, linkage studies and genome-wide association studies have proved the role of genetic factors as risk factors for the development of ischemic stroke. This research had been initiated approximately 1.5 decade ago, and three major blocks of research approaches can be separated. In the first wave of trials the classic susceptibility markers discovered originally in relation of other diseases like cardio vascular disease, affecting different pathways, such as the inflammatory and haemostatic system, homocysteine metabolism and the renin–angiotensin-aldosterone system were tested also for stroke. Very numerous reports had been presented about the “classical” factors in different populations; the articles used biobanks with just a couple of hundreds or even less samples. In the second and still ongoing group, which is a kind of bridge between the two other blocks of studies, the spectrum of candidate genes rapidly increased, functional variants of genes or genomic regions still discovered primarily in relation to other diseases, were tested on larger stroke biobanks of clinically better stratified patients, large number of these alleles were originally discovered by array based genome-wide association studies. The third period of research had been started with the dramatic spread of the direct array screening of the large biobank samples; this approach represent still a huge significance in the further progress. What we learned is that the careful stratification of patients is critical and obligatory; the susceptibility alleles are often shared; not all susceptibility factors, that associate with clinical traits that can be itself a risk factor to stroke (like increase of

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triglycerides), are necessarily represent susceptibility for stroke. The studies still mainly focus on large and small vessel associated stroke, and the knowledge on other types of stroke which represent much smaller population samples are still very scarce. Albeit some susceptibility allele tests are on the palette of some direct-to-consumer (DTC) companies, the clinical utility and clinical validity of these test results still does not support their use in the patient care.

OVERVIEW OF STROKES

According to the definition of the World Health Organization (WHO) stroke is a suddenly developing temporary, or in unfavorable cases permanent damage of the brain. This definition has been used for more than 20 years in the clinical practice to describe this heterogeneous condition. [140;142] It can affect both males and females at any time of life; however, it is more prevalent in males over 45 years of age. [27]

The disease can be classified into two subgroups: hemorrhagic and ischemic stroke. Ischemic stroke comprises 80-85% of all stroke cases; 15-20% of the cases are hemorrhagic, like intracerebral or subarachnoid. Various pathophysiological mechanisms can result in ischemic stroke. Ischemic stroke can be a result of atherothrombosis (75% of cases) or embolism (25% of cases) leading to occlusions in arteries. However, 30% of ischemic strokes can be form from other causes, like systemic hypoperfusion.

Acute ischemic stroke is the leading cause of death and the most commons cause of disability in the developed countries [111]. In incidence, prevalence, and mortality of stroke huge differences can be seen among countries worldwide. The incidence rates of stroke in the US have been proven to be the highest in the world with 795,000 cases annually; 150,000 deaths occurred in 2011. The prevalence rate was approximately 5.8 million, of which 87% was ischemic stroke. In Japan, stroke is also a major public health problem; the prevalence rate of stroke was about 1.4 million in 2005, of which 61% was ischemic. [144] In Europe, stroke has similar epidemiology rate; the incidence of stroke is less in European populations than in the US. The observation of an epidemiological review there are large geographical variations in the incidence of stroke, which show decreasing gradient from eastern to western European countries: among the examined populations, the incidence of stroke was the highest in the Russian populations: 600,000 annually as against 210,000 per year in French in 2007. [126]

STRATIFICATION OF ISCHEMIC STROKE

The stringent classification of subtypes of ischemic stroke is undoubtedly critical from the viewpoint of its etiology. It can be divided into three major subgroups depending on the *pathogenic* mechanisms: thrombotic, embolic and hemodynamic stroke, which is a broadly accepted stratification of ischemic stroke. This classification is based on results received by imaging examinations. These groups can overlap and are not necessarily specific enough. However, the exact determination of subgroups cannot be ignored in point of prevention and its treatment.

The first major *clinical* classification was introduced in 1991 with the Oxford Community Stroke Project [8], which was followed by a more widely accepted one in 1993. This was based on the most common pathophysiological mechanisms involved in the development of stroke: Trial of Org10172 in Acute Ischemic Stroke (TOAST). The system comprises five subgroups: 1) large-vessel atherosclerotic type, in which patients have cortical or cerebellar lesions and/or brainstem infarcts or subcortical hemispheric infarcts greater than 1.5 cm in diameter on magnetic resonance imaging (MRI), with or without cerebral cortical impairment or brainstem or cerebellar dysfunction; 2) small-vessel occlusion type: patients with one or more subcortical hemispheric or brainstem infarcts with a diameter <1.5 cm on MRI, with one of the features of the traditional clinical lacunar syndrome and without cerebral cortical dysfunction 3) cardioembolic subtype comprises patients with arterial occlusions presumably due to an embolus arising in the heart; 4) stroke with other determined etiology, which includes patients with rare causes of stroke like hematologic disorders, nonatherosclerotic vasculopathies and hypercoagulable states; 5) stroke with unidentified etiology, in which the cause of stroke cannot be determined. [3]

RISK FACTORS FOR STROKE

Stroke is a multifactorial disease. Environmental factors can have crucial role in the development of the disease. Several risk factors were identified in the development of ischemic stroke, which were categorized as potentially modifiable and non-modifiable ones. [18;68;116]. Thus, age and gender were enrolled into non-modifiable risk factors; besides previous stroke in case history, which increases considerably the probability of subsequent cerebrovascular event, can also be enrolled into non-modifiable risk factors.

Hypertension, diabetes mellitus, obesity, elevated triglyceride- and cholesterol levels, smoking, exaggerated alcohol consumption and presence of any cardiovascular events like atrial fibrillation, myocardial infarction, carotid stenosis are categorized as modifiable risk factors in this classification. These factors proved to be unambiguous risk factor for the development of disease, so control of these risk factors compose part of primary prevention strategies. Recently, additional possible risk factors like several naturally occurring variants within the genome came into the focus of epidemiological studies. So far a significant progression could be observed in the identification of genetic variants in the background of stroke, and other cerebro- and cardiovascular disorders. [5;23;29;42;87]. Investigation of these variants provides novel approach to the knowledge of pathogenesis of conditions.

MONOGENIC DISORDERS ASSOCIATED WITH ISCHEMIC STROKE

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leucoencephalopathy (CADASIL)

CADASIL is a heritable stroke associated disease caused by mutations in the NOTCH3 gene [57]. However, not-all patients with this clinical phenotype have a NOTCH3 mutation [96]. The clinical phenotype comprises multiple lacunar strokes and transient ischemic

attacks, migraines with aura and ultimately, cognitive impairment due to vascular dementia with usually onset in the third to sixth decade [24]. A relatively unique but not pathognomic diagnostically important feature of the disease is the bilateral white matter involvement of the anterior temporal lobes and the external capsules [6;93].

The gene encodes cell-surface receptor which is expressed on vascular smooth muscle cells. The receptor is a heterodimer with a large extracellular domain containing epidermal-growth-factor-like repeat domains. Mutations usually occur in the 2 to 24 exons of the gene, resulting in a change of the number of cysteine residues [58;106]. Despite that the mutation spectrum being broad, the genotype seems to have no major impact on the phenotype. The underlying mechanism of the disease is poorly understood. The most specific and sensitive diagnostic method is skin biopsy. Multiple studies showed that, patient despite the nature of their NOTCH3 mutation, had granular osmiophilic deposits within their vascular basal lamina [114;139].

A recent 7-year long longitudinal study of 25 NOTCH3 mutation carrier CADASIL-patients, came to the realization: that the cognitive decline is rather to be associated with lacunar strokes, microbleeds and increased ventricular volume than white matter changes shown on MRI [67]. A recent monozygotic twin study pointed out the importance of environmental factors in the disease. The twin who smoked and had a sedentary life style suffered a stroke 14 years sooner than the twin with a healthy life style [89].

Cerebral Autosomal-Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL)

CARASIL is a cerebral small-vessel arteriopathy transmitted in an autosomal recessive manner. Typical clinical features are normotension, alopecia, strokes and eventually dementia with onset in the first 3 decades [30]. The genetic background of the disease, are mutations affecting the HtrA serine protease 1 (HTRA1) gene [40].

Fabry's Disease

Fabry's disease is an X-linked metabolic disorder caused by the deficiency of lysosomal enzyme α -galactosidase A. The insufficient enzyme activity is associated with accumulation of glycosphingolipids mainly in the heart, kidneys, eyes, skin and the vascular system. Clinical symptoms include acroparesthesia, anhidrosis, corneal opacities, cataracts, angiokeratomas, and renal, cardiac failure with a typically childhood or adolescent onset [28]. Cerebrovascular events occur from both large and small vessel disease, frequently affection the regions supplied by the posterior circulation [36;112].

However, only small vessel involvement is associated with white matter changes. Manifestations of Fabry's disease been also reported in heterozygote female carriers of the mutation, with clinical features ranging from asymptomatic, mild manifestations to rare cases showing severe disease as in affected males [22]. The disease can be confirmed with enzyme activity measurement however, the method in women is less reliable due to the skewed inactivation of the X chromosome.

The prevalence of the disease was surprisingly high in young stroke patients according to a German study, suggesting 4.9% of all cases to be GLA mutation being the underlying cause of cryptogenic ischemic stroke in young men [112]. Recent US studies however, showed differ. The Stroke Prevention in Young Men Study observed Fabry's disease in 0.18% of all strokes, and 0.65% of cryptogenic strokes [151].

Therapy of the disease involves enzyme replacement therapy. Recent studies showed that the enzyme replacement therapy, according to a five-year data report, proved to be significantly reducing the pain and life quality of Fabry patients. Treatment also seems to act favorable in cardiac parameters, including left ventricular mass reduction and an increase in midwall fraction shortening [81].

Sickle-Cell Disease

Sickle-cell disease is the most common cause of ischemic stroke in children [128]. The underlying cause of the disease can be a homozygous state for hemoglobin S, or a compound heterozygous state associated with other types of hemoglobinopathies such as hemoglobin C or α -thalassemia [95]. Hemoglobin S carriers have a point mutation in the β -globin chain of hemoglobin, causing the hydrophilic amino acid glutamic acid to be replaced with the hydrophobic amino acid valine at the sixth position.

This change, under low-oxygen condition promotes a non-covalent polymerization (aggregation) of hemoglobin thus creating the critical component of the pathogenesis of the disease, namely abnormal vessel-red blood cell interaction with increased thrombus formation [128]. Homozygous carriers have approximately 25% chance of developing stroke before the age of 45 [94]. The incidence for stroke is highest between 2-5 years and stroke recurrence is common.

Clinically manifested strokes are usually associated with large vessel involvement, and are typically located to the borderzone regions [99]. The risk of stroke in sickle-cell disease is strongly affected by the genotype of the affected patients. Polymorphisms in the fetal hemoglobin gene, in cell-adhesion molecules VCAM and P-selectin or variations in the interleukin 4 receptor gene may greatly modify the clinical outcome of the disease [25;49;120;138].

Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-Like Episodes (MELAS)

MELAS is a syndrome characterized by developmental delay, hearing loss, short stature, seizures, vomiting, diabetes, migraine and cognitive decline [23]. The onset of the disease is usually in childhood initially characterized by tonic-clonic seizures, headache and vomiting. MELAS is associated with several mitochondrial mutations [78;100].

The most frequent mutation is a adenine to guanine change at position 3243 in the tRNA^{Leu}(UUR). The second most common (T3271C) is the genetic background in 10% of all cases. In MELAS the cortex is almost always involved in stroke like episodes, and lesions do not limit to vascular territories, since the underlying cause is an energetic imbalance instead of vascular occlusion [30].

Homocystinuria

Homocystinuria is a mostly recessively inherited enzyme deficiency related disease characterized by high levels of plasma homocysteine and homocystinuria. Clinical features of the disease are mental retardation, atraumatic lens dislocation, Marfan-like skeletal abnormalities and thrombotic events including stroke [23]. Most common cause of the disease is a deficiency of cystathion beta-synthase (CBS); however, the mutation spectrum is broad [66]. Half of untreated patients have thrombotic events by the age of 30 and some of these cases result in stroke [88]. Mechanism of stroke can be either thrombotic, small vessel related or due to arterial dissection [41;62]. Concurrent Leiden mutation raises thrombosis risk [74]. B6 vitamin therapy responding patient have generally better prognosis, and milder symptoms compared to non responders [154].

Moyamoya Disease

Moyamoya disease is a chronic progressive, occlusive, cerebrovascular arteriopathy characterised by bilateral stenosis of distal carotid vessels with frequent feature of telangiectic vessel changes of the circle of Willis [122]. The disease is rare in non-Asian population however, its prevalence in Japan is estimated 3/100000 [122].

The disease may manifest both in childhood with clinical symptoms of transient ischemic attacks, strokes and epilepsy [23], or in adult with symptoms raising from rupture of telangiectic vessels developed during childhood [7]. 10% of all cases occur as familial cases, however, the pattern of hereditary of the disease is not well understood [31]. Both autosomal dominant and polygenic mode of transmission with incomplete penetrance have been suggested [23;122]. The disease has been mapped to loci for the ch3p (MYMY1), variations in the RNF213 gene on ch17q25 confer susceptibility to (MYMY2) and ch8q23 (MYMY3), while (MYMY5) is caused by mutation in the ACTA2 gene on ch10q23.3.

Finally, an X-linked recessive syndromic disorder characterized by the Moyamoya phenotype, short stature, hypergonadotropic hypogonadism and facial dysmorphism has been identified in patients of three unrelated families (MYMY4). The deleted region identified on chXq28 and included exon 1 of the MTCP1/MTCP1NB gene and the first three exons of the BRCC3 gene [23;53;86;117;153]. Moyamoya may be also secondary or associated with other disease like: sickle-cell anemia, pseudoxanthoma elasticum, hemoglobinopathies, Down's syndrome, neurofibromatosis type I, William's syndrome, Graves disease and neurofibromatosis type I [23;122;125].

Marfan's Syndrome

Marfan syndrome is an autosomal dominantly inherited disease affecting the musculoskeletal system, cardiovascular system and the eye [59]. Other clinical features of the disease consist of chest deformities including both pectum excavatum and carinatum, scoliosis, lens deformities including luxation of the lens, arachnodactylia, generally long limbs (upper to lower segment ratio <0.86, or arm to height ratio >1.5), however the most serious complication is the dilation and/or dissection of the aorta [2;20;23;59].

Table 1. Classification of stroke subtypes

Classification	Reference
Trial of Org10172 in Acute Ischemic Stroke (TOAST)	[3]
large-artery atherosclerosis (embolus/thrombosis)*	
cardioembolism (high-risk/medium-risk)*	
small-vessel occlusion (lacune)*	
stroke of other determined etiology*	
stroke of undetermined etiology	
two or more causes identified	
negative evaluation	
incomplete evaluation	

*Possible or probable depending on results of ancillary studies.

Genetic background of the disease are mutations in the very large gene of FBN1 (65exons), encoding fibrillin-1, an extracellular matrix protein.

Diagnosis of the disease is usually a clinical one [20], since the mutation spectrum is broad and direct sequencing of the gene is rather expensive and laborious [91]. There is increased evidence that fibrillin-1 shares homology with TGF binding proteins, thus TGF might play a key role in the development of the disease [59;90]. Ischemic strokes and subdural hematomas both can be both manifestations of cerebrovascular complications of the disease; however the underlying mechanism is not well understood. A retrospective study associated these complications with embolisms, originating from the heart, in particular mitral valve prolapse, atrial fibrillation and prosthetic valves. The study involved 513 patients and found no association with aortic disease or cerebral vessel dissection [150]. However, others observed association of cerebrovascular events associated with vessel dissection [119;124], and there have been reports of strokes in patients taking chronic anticoagulant therapy and perioperative embolic cases in patients undergoing aortic replacement surgery [33].

Ehlers-Danlos Syndrome

Ehlers-Danlos syndrome is a group inherited disease mostly affecting the connective tissues and caused by mutations in genes responsible for coding collagen (Type-I or Type-III). From the different types of the disease, the autosomal dominantly inherited Type-III is the one where cerebrovascular complications like large and medium-sized artery rupture, intracranial aneurysms and arterial dissection are common [92;119]. Phenotypic features of the disease include characteristic facial appearance (big eyes, lobeless ears, thin nose and lips, sunken cheeks), small stature with a slim body, thin-translucent skin with visible veins specially over the chest and abdomen, and due to the fragile vessels, easy bruising and rupture of other organs like the uterus or the intestines [9].

The genetic background of the disease is mutations in the COL3A1 gene (encoding collagen Type III) with a broad mutation spectrum [105]. Neomutations are common and only 50% of all cases have a positive family history [105]. In a clinical study of 419 patients, 11% had neurovascular complications [105].

PERIODS IN THE STROKE SUSCEPTIBILITY RESEARCH

The Classic Genetic Risk Factors and the Stroke

At the beginning of genetic background's research of stroke, examinations of single genetic variants had been carried out, which might be involved in the possible pathogenic mechanisms of this condition. This period, which can be regarded as the first wave in the stroke research, was otherwise the beginning of the susceptibility allele research for numerous other multifactorial diseases, including ischemic heart disease, type 2 diabetes, and metabolic syndrome, as well. Among these, several polymorphisms of genes encoding enzymes involved in the renin-angiotensin system, nitric-oxide production; and of genes influencing hemostasis, homocysteine- metabolism are shown in Table 2 and Table 3.

Disturbance of balance of hemostasis can lead to increased aggregation of blood platelets, thrombus formation, which may results from defects of genes encoding components of coagulation cascade like factor II, V, XIII and fibrinogen promoting development of several cardio- and cerebrovascular phenotype. Activated factor V acts as a cofactor for the conversion of prothrombin to thrombin.

In the F5 gene, c.1691G>A transition (p.Arg506Gln) was first identified by Bertina and co-workers in a family with thrombophilia in 1994. [10] The c.1691G>A alteration has been reported to be associated with resistance to degradation by activated protein C. Since then, numerous studies were engaged in examining this variant in several conditions in correlation with thromboembolism, whose results were inconsistent regarding the pathological role of this alteration. [4;15;19;109]

Table 2. Genetic-association studies: genes and their polymorphisms identified in the background of ischemic stroke

Gene	Polymorphism	Reference
hemostasis		
II. factor (prothrombin) (+176930)	c.20210G>A	[14;21;77;108]
V. factor (Leiden) (*612309)	p.Arg506Gln	[4;10;14;15;19;109;110;134]
XIII. factor (+134570)	p.Val34Leu	[14;16;32]
renin-angiotensin system		
ACE (+106180)	insertion/deletion	[12;121;133;134;157]
AT1R (*106165)	c.1166A>C	[11;113;130;132]
nitric-oxide-synthase system		
eNOS (+163729)	p.Glu298Asp	[50;129;143]
homocysteine metabolism		
MTHFR (*607093)	c.677C>T	[21;75;131;135]
	c.1298A>C	
lipid metabolism		
APOE (+107741)	ε4, ε3, ε2	[17;26;60;63;76;134;141]

Abbreviations: ACE: angiotensin-converting-enzyme; APOE: apolipoprotein E protein; AT1R: angiotensin-receptor; eNOS: endothelial nitric-oxide synthase; Gln: glutamine; Leu: leucine; MTHFR: 5,10-methylenetetrahydrofolate reductase; OMIM: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM>.

Table 3. Functional SNPs in the background of ischemic stroke

Gene	Polymorphism	Reference
lipid metabolism		
APOA5 (*606368)	g.-1131T>C, c.1259T>C	[43;52;71;72;102;103;136]
	c.56C>G, c.IVS3+476G>A	
APOCIII (*107720)	g.-2854T>G, g.-455C>T	[1;137;145;146]
	g.-482C>T, c.3238C>G	
LPL (*609708)	p.Asn291Ser	[44;51;82;149]
GCKR (*600842)	c.1337C>T	[97]
MLXIPL (*605678)	rs17145738, rs3812316	[98]
GALNT (*602274)	rs4846914	[97]
signal transduction		
PDE4D (*600129)	6 SNPs, haplotypes	[35;69;80;85;155]
inflammation		
ALOX5AP (*603700)	7 SNPs, haplotypes	[45;46;69;85;156]

Abbreviations: ALOX5AP: arachidonate 5-lipoxygenase-activating protein; APOA5; CIII: apolipoprotein A5; -CIII proteins; Arg: arginine; Asn: asparagine; LPL: lipoprotein lipase; OMIM: <http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM>; PDE4D: phosphodiesterase 4D.

According to achievements of a large-scale epidemiologic assessment, carriage of 1691A allele was not associated with an increased risk of stroke. [110] However, statistically significant association was found between the presence of c.1691G>A transition and ischemic stroke in a meta-analyses. [14] Szolnoki and co-workers found that c.1691G>A alteration does not confer risk for stroke alone, but in combination with other unfavorable factors like hypertension or diabetes mellitus can increase the relative risk of ischemic stroke. [134]

The most extensively studied alteration of prothrombin (factor II) gene is c.20210G>A, which was first described in 1996. [108] This sequence variation is associated with elevated prothrombin levels. Several studies found a statistically significant association between ischemic stroke and this substitution [21;77]; the data of a meta-analysis also suggest that c.20210G>A variant is a risk factor for ischemic stroke. [14]

The susceptibility role of p.Val34Leu substitution in factor XIII encoding gene remains ambiguous according to the findings of association studies. [16;32] The meta-analysis of Casas and colleagues suggest that p.Val34Leu variant does not confer increased risk for development of stroke. [14]

The c.455G>A alteration of fibrinogen encoding gene have been found to occur more frequent in homozygous form in large-vessel stroke subgroup. [63] Carrying the rare allele causes elevated fibrinogen levels, which confer susceptibility to ischemic stroke via prothrombotic mechanisms. Similar relationship was found for other variants of fibrinogen, however, only in female patients, which mechanism is unclear yet. [13]

The renin-angiotensin system regulates the hypertension and water balance. When hypotension occurs, renin is secreted from the kidneys, which protease cleaves angiotensinogen converting angiotensin I. This intermedier forms into angiotensin II catalyzed by angiotensin converting enzyme (ACE). This enzyme encoding gene located on chromosome 17 is one of the most intensively studied candidate gene in association with ischemic stroke. Cambien and colleagues have first described the insertion/deletion

polymorphism (I/D) of the gene in myocardial infarction. [12] Six years later, a meta-analysis reported significant positive association between deletion polymorphism of ACE gene and ischemic stroke. [121] The results of a large, prospective study indicate that the ACE I/D polymorphism is not associated with subsequent risk of stroke. [157] In a Hungarian population ACE D/D confers risk for development of stroke. [133;134] Subsequent studies tended to variant of I type of angiotensin II receptor encoding gene (AT1R c.1166A>C). The AGTR1 has multiple effects, both cardiac and systemic, mediating vasoconstriction, cellular hypertrophy and catecholamine release. In a Caucasian population weak association was found between carrying 1166C allelic variant and stroke. [11;113] According to the results of Szolnoki and colleagues 1166C allelic variant represents susceptibility to the disease in hypertensive smokers. [130] Co-occurrence of ACE I/D and AT1R c.1166A>C variants was found to confer risk for small-vessel occlusion type in Hungarian population. [132] Several studies have investigated ACE I/D polymorphisms in association with numerous conditions, but its role remains controversial. It has been delineated that ACE D/D polymorphism contributes to the development of atherosclerotic mechanisms, which can be modified by gene-gene and/or gene-environmental interactions.

The nitric-oxide-synthase system is an important regulator of integrity and growth of vascular endothelium and is an ubiquitous molecule responsible for the maintenance of normal endothelial function. The key enzyme is the endothelial nitric-oxide-synthase (ENOS), which converts L-arginine to L-citrulline generating nitric-oxide (NO) in the arteries, and has role in the vasodilatation. Under pathologic conditions superoxide has arisen instead of NO in the cells, which can initiate atherosclerotic process. Defects in the gene encoding ENOS was found in the background of pathological functions. In 1996, Wang and colleagues identified a functional variant (enos4a/b) of ENOS encoding gene in association with coronary artery disease (CAD) first in the literature. [143] Since then there are few reports on candidate variants in ENOS gene in association with ischemic stroke. Howard and colleagues found that promoter region polymorphism (-786T>C) of ENOS gene is associated with ischemic stroke susceptibility in young black women. [50] Another SNP in this gene (c.894G>T) has been shown not to be a susceptibility factor for ischemic stroke, however, co-occurrence with other SNPs like ACE D/D, MTHFR c.677C>T could increase stroke risk. [129]

Hyperhomocysteinemia caused by pathological homocysteine metabolism have been shown to be associated with increased risk for several cardio- and cerebrovascular events. Elevated homocysteine levels can be resulted from reduction of 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme activity, which catalyses the methylation of homocysteine. Extraordinary, complete absence of MTHFR enzyme was also observed. Variants in MTHFR gene (c.677C>T, c.1298A>C) can lead to deficiencies in the enzyme activity. The c.677C>T alteration was found to represent an important risk for cerebral events. Examinations of these polymorphisms revealed no association between carriage of 677T or 1298C alleles and development of stroke. [21;75] However, Szolnoki and co-workers stated later that co-occurrence of these two alleles confer susceptibility to ischemic stroke both in hetero- and homozygous form. [135] A case-control study demonstrated that 677T allelic variant is more prevalent in ischemic stroke patients in co-occurrence with AT1R c.1166A>C variant; and they confer together increased risk for stroke. [131]

Apolipoprotein E, the main apoprotein in chylomicrons, serves as a ligand for cellular receptors on liver and peripheral cells and interacts with proteoglycans on the endothelium

guiding lipoproteins to lipases. The APOE gene located on chromosome 19 is polymorphic with three codominant alleles $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ defining six genotypes. [141] Carriers of the $\epsilon 2$ alleles have been associated with lower total and LDL-cholesterol levels, as well as elevated triglyceride levels. Carriers of the $\epsilon 4$ allele have increased levels of both total and LDL-cholesterol compared to $\epsilon 3/\epsilon 3$ homozygotes. [26] A dozens of studies have delineated that APOE is an important predicting factor for the development of ischemic stroke. [17;63;76] At 10-year follow-up study by Kathiresan, the genotype data confirmed this association. [60] Szolnoki and co-workers observed that APOE $\epsilon 4$ allele alone represents a minor genetic risk factor for ischemic stroke. However, co-occurrence of APOE $\epsilon 4$ allele and traditional risk factors like hypertension, diabetes mellitus, smoking and alcohol consumption represents considerably elevated risk for ischemic stroke. [134]

Further Search for the Functional SNPs in Stroke

In the next period of the stroke research there was a great step ahead, as the profile of the functional variants as possible susceptibility genes was increased very rapidly. Increased concentrations of plasma lipids, triglyceride and cholesterol levels were found to be independent risk factors for cardio- and cerebrovascular disorders. [47;48;73] Plasma triglyceride levels are determined by numerous genes and their interactions with the environment. Search for the genetic variations affecting plasma triglyceride concentrations is still ongoing, several loci were identified so far include APOAI/CIII/AIV, APOA5 and LPL. [60;148]

The apolipoproteins are lipid binding proteins, involved in the plasma transport of lipids. Genetic variations influencing their protein structure or synthesis may affect lipid metabolism resulting in progression to cardio- and cerebrovascular disorders. A gene cluster was identified on chromosome 11, which contains APOAI, APOCIII, APOAIV genes. [37] Apolipoprotein CIII (APOCIII) encodes a protein of 79 amino acids that is synthesized in the liver and the intestines and which is an essential constituent of circulating particles rich like VLDL and chylomicrons. [56] Several polymorphisms of apolipoprotein CIII (APOCIII) have been associated with hypertriglyceridemia. [137] Carriers of the G allele of the c.3238C>G polymorphism of APOCIII 3'-UTR were reported to have higher plasma triglyceride concentrations, as do individuals with two minor alleles, -482C>T and -455T>C of the APOCIII promoter. [145;146] Since ApoCIII inhibits the hydrolysis of triglyceride-rich particles by lipoprotein lipase, its overexpression leads to hypertriglyceridemia. [56] Only a few studies were engaged in exploring the role of APOCIII gene SstI polymorphism in the development of ischemic stroke. Aalto-Setälä and colleagues found a negative association between APOCIII polymorphism and ischemic stroke. [1]

The apolipoprotein A5 (APOA5) gene is located near the APOAI/APOCIII/APOAIV cluster encoding 366 amino acids. [101] The mature APOA5 protein is synthesized in the liver and thereafter is secreted into the plasma. [147] Evidence supports that APOA5 plays a central regulatory role in triglyceride metabolism through the inhibition of hepatic VLDL production or by guiding VLDL and chylomicrons to proteoglycan-bound lipoprotein lipase. [83;84] Furthermore, APOA5 has been described to independently influence plasma triglyceride levels in an opposing manner than APOCIII. [115] Around 40 polymorphisms of the APOA5 gene have been identified since the discovery of the gene. [52;103;136] These

natural variants may alter the function of the protein. The most common natural variants, -1131T>C (rs662799) in the promoter region, g.1259T>C (rs2266788) in 3'UTR region, c.56C>G (with Ser to Trp amino acid change in the signal peptid, rs3135506), and intronic g.IVS3+476G>A (rs2072560) have been repeatedly reported to associate with elevated triglyceride levels. [101-103] Some of them were also found to be independent risk factors for several conditions associated to triglyceride metabolism. The carriage of -1131C and g.IVS3+476A mutant alleles have been established to confer increased risk for all subtype of ischemic stroke. [43;71] 56G allele was found to associate with only large-vessel stroke in Hungarian subjects. [72] In contrary, 1259C variant did not represent increased risk for the disease. [71] The most common SNPs of APOA5 gene are in strong linkage disequilibrium and constitute haplotype variants (APOA5*2,*3,*4,*5). Among these APOA5*2 was ascertained to be a risk factor for ischemic stroke. Otherwise, APOA5 is an example for the shared susceptibility nature: the naturally occurring variant and haplotypes have been found to confer susceptibility to other diseases like metabolic syndrome. [64;65;70]

Another important factor of triglyceride metabolism is the lipoprotein-lipase (LPL), which catalyses the hydrolysis of chylomicron and VLDL-associated triglycerides with ApoCII cofactor. [44] Approximately 100 polymorphisms have been described in the LPL gene. Most of these variants can lead to decreased LPL-activity, which increase plasma triglyceride levels and the risk of atherosclerosis. [82] Huang et al. found that the p.Asn291Ser alteration has significant influence on serum levels of triglycerides, but this LPL polymorphism does not contribute greatly to the overall risk of ischemic stroke. [51] The results of a subsequent study suggest that the p.Asn291Ser substitution is not associated with ischemic stroke in men but that it possibly confers a two-fold risk in women. [149]

The cAMP-specific 3',5'-cyclic phosphodiesterase 4D (PDE4D) is an important regulator of proliferation of smooth muscle cells and macrophages by degrading a key signaling molecule, cAMP. Defects of the protein results in accumulation of cAMP in various cell types like monocytes, and T-lymphocytes. It can inhibit immune functions like proliferation and cytokine secretion, which could generate atherosclerosis and plaque instability. The gene located on chromosome 5 encoding PDE4D was identified by Gretarsdottir in 2003. [35] Among the 260 SNP examined by the authors, six have been found to be associated with stroke. Besides, analyses of haplotypes showed significant correlation between certain haplotypes and ischemic stroke, as well. Since then 16 replication studies have been published on the association between SNPs in PDE4D and ischemic stroke in different populations, which presented various results. Some of them confirmed the previously described positive association, but there are studies, which disputes the presence of association. [69;80;85;155] The inconsistent findings among the studies can be originated in differences between population sample and ethnicity.

Beside PDE4D, arachidonate 5-lipoxygenase-activating protein encoding gene (ALOX5AP) came into the center of interest of association studies. The gene encodes a proinflammatory mediator, which is implicated in the pathogenesis and progression of atherosclerosis. Several polymorphisms and haplotypes were identified in the ALOX5AP gene, which have been shown to confer increased risk for stroke in Icelandic population. [46] This result was confirmed in German and Scottish population. [45;156] However, some studies found no association between the presence of polymorphisms in ALOX5AP and clinical phenotypes. [69;85] Investigation of functional consequences of these variants has just begun.

Interestingly, the spread of the genome-wide association studies reached first other diseases, like the Parkinson's disease, myocardial infarction, inflammatory bowel disease including, or even relatively rare conditions like the macular degeneration, the stroke disease was involved secondarily, as several SNPs discovered originally in relation with other diseases, were also tested in stroke biobanks. Thus, variant discovered in genome-wide association studies, focused on alleles involved in triglyceride metabolism, a variant (rs780094) of the glucokinase regulatory protein (GCKR), which associate with increased triglyceride levels was also investigated. [60;148] The GCKR gene, located on chromosome 2 encodes a protein of 625 amino acids. GCKR protein inhibits glucokinase in liver and pancreatic cells by binding the enzyme non-covalently to form an inactive complex. Jaromi et al. investigated the GCKR c.1337C>T (rs1260326) variant in a case-control study on stratified stroke population. No association could be detected between GCKR alleles and triglyceride levels or with the susceptibility to stroke. [55]

Single nucleotide polymorphisms localized at the GALNT2 and MLXIPL/TBL2 loci have also been found to be associated with increased triglyceride level by GWA studies. [60;148] The rs4846914 is an intronic variant of the UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GALNT2) gene, and its minor G allele was reported to associate with higher triglyceride concentrations. The GALNT2 gene is responsible for the transfer of an N-acetyl galactosamine to the hydroxyl group of a serine or threonine residue in the first step of O-linked oligosaccharide biosynthesis. A Hungarian case-control study evaluated the association of GALNT2 rs4846914 variant with triglyceride levels, and explored their possible contribution to the development of ischemic stroke.

No positive association was detected either with triglyceride levels or with susceptibility to the disease. [55] The major alleles of rs17145738 and rs3812316 variants within the MLXIPL/TBL2 region have been found to be associated with increased triglyceride levels. [60;148] MLXIPL encodes a transcription factor, MXL interacting protein-like which plays a role in the activation of carbohydrate response element motifs in the promoter region of genes involved in lipogenesis and triglyceride synthesis, and regulates lipogenesis and glucose utilization in the liver. TBL2 encodes transducin beta-like 2 protein which is thought to be involved in intracellular signaling. Polgar and co-workers examined the possible susceptibility nature and the effect of rs17145738 and rs3812316 variants on triglyceride levels. The results showed that presence of these variants did not result in elevated triglyceride levels and did not confer risk for ischemic stroke. [107]

Direct Genome-Wide Association Studies in Ischemic Stroke Biobanks

The first GWAS directly done in ischemic stroke patients was published in 2007 [79]; in this year the GWAS was announced as breakthrough of the year by the Science magazine. [104] The GWA studies in association with ischemic stroke are summarized in *Table 4*. Matarín and colleagues analyzed 278 ischemic stroke patients and 275 controls for more than 408,000 unique SNP using Illumina Infinium Human-1 and HumanHap300 assays. The ischemic stroke subtypes were allocated according to the specified TOAST classification.

Table 4. Genome-Wide Association Studies in stroke

Population	Sample size	Sample size in replication	Number of SNPs examined	Number of SNPs examined in replication	Positive association		Platform	Reference
					Gene	Variant		
Caucasian	19,602 individuals	3656 cases, 3613 controls	2,194,468	2	near NINJ2 and WNK1 genes	rs11833579 rs12425791	Affymetrix and Illumina	[54]
Japanese	6,341 individuals	705 cases, 3426 controls	520,000	100	CELSR1 (*604523)	rs6007897 rs4044210	Affymetrix	[152]
Caucasian	1,661 cases, 10,815 controls	2224 cases, 2583 controls/ 2327 cases, 16760 controls	310,881	120/2	near PITX2 (*601542)	rs2200733 rs10033464	Illumina	[34]
US	249 cases, 268 controls	-	408,000	-	IMPA2 (*605922) KCNIP4 (*608182) KCNK17 (*607370)	rs7506045	Illumina	[79]

Abbreviations: CELSR1: cadherin EGF LAG seven-pass G-type receptor 1; IMPA2: inositol(myo)-1(or 4)-monophosphatase 2; KCNIP4: Kv channel interacting protein 4; KCNK17: potassium channel, subfamily K, member 17; NINJ2: Nerve injury-induced protein 2; PITX2: paired-like homeodomain 2.

The results showed hundreds of nominally statistically significant associated markers, of them notable candidate loci are IMPA2 on chromosome 18, KCNIP4 on chromosome 4, KCNK17 gene chromosome 6, which are involved in the phosphatidylinositol signaling pathways and potassium transport, respectively. [79]

In 2008, Gretarsdottir described the results of a well-powered GWA study with 1,661 ischemic stroke patients from the Icelandic population classified according to TOAST and 10,815 controls. The authors used Illumina Infinium Human-1 and HumanHap300 chips for genome-wide genotyping containing more than 310,000 SNPs.

From these, 20 SNPs were selected for replication. Of those, rs2200733 and rs10033464 in the transcriptional activator encoding PITX gene on chromosome 4q25 segment have been found to associate significantly with development of ischemic stroke, especially for stroke due to cardioembolism (cardiogenic stroke). [34] Some variants identified in the gene have been found to be associated with Axenfeld-Rieger syndrome previously. [123;127]

A Japanese GWAS, which called 6,341 participants (992 IS patients and 5,349 controls) to the study, analyzed more than 520,000 SNP applying GeneChip Human Mapping 500K Array by Affymetrix.

After receiving results Yamada and colleagues selected 100 SNPs for replication study. According to the results polymorphisms in the CELSR1 gene on chromosome 22 have been found to be susceptibility gene for ischemic stroke. The CELSR1 gene encodes a transmembrane G protein-coupled receptor. [152]

Combined analysis of genome-wide association study data generated from four large cohorts (Framingham Heart Study, Rotterdam Study, ARIC and Cardiovascular Health Study) was carried out by Ikram et al. The cohorts include 19,602 applicants, of whom 1164 suffered from one of the ischemic stroke subdivided into atherothrombotic and cardioembolic subtypes.

The genotyping was performed with different platforms (Affymetrix GeneChip SNP Array 6.0, GeneChip Human Mapping 500K Array and 50K Human Gene Focused Panel; Illumina HumanCNV370-Duo and Infinium HumanHap550 3.0). Two of the analyzed SNPs, rs11833579 and rs12415791 on chromosome 12 near NINJ2 and WNK1 genes, are implicated into replication study, which revealed positive association between carrying minor alleles and ischemic stroke, in particular, atherothrombotic stroke subtype. [54]

The spectrum is still growing, and the major achievements can be found at the website of the National Institutes of Health (<http://www.nih.gov/>).

Beside its huge power and impact, the GWA studies have also known limitations. In a few cases individuals involved into the study derived from one population. The validation of their findings will be required in other independent ethnic groups.

In certain replication studies, the number of subjects was too small and functional relevance of the identified polymorphisms remains unclear. So further genotyping, detailed functional and epidemiological studies in a greater population are required to identify the exact role and associations of these genes and gene variants with conditions.

CONCLUDING REMARKS

In the past almost 2 decades a significant progression has been made in the stroke susceptibility genetics. Clear imbalance can be observed: while some major types of the diseases, like the large- and small vessel pathology associated types are intensively investigated, the less frequent forms escaped the focus. It is clear now that the careful stratification of patients is critical and obligatory for research purposes. Several genes or susceptibility alleles are often shared.

It is also clear, that some susceptibility factors that associate with clinical traits that can be itself a risk factor to stroke, as they associate with increased triglycerides, are necessarily represent susceptibility for stroke. Since the role of abnormalities of several laboratory, and biochemical parameters is uncertain in the development of ischemic stroke, growing knowledge about their nature will help the understanding also their role in stroke.

Albeit some susceptibility allele tests are offered on the palette of some direct-to-consumer companies, the clinical utility and clinical validity of these test results still does not support their current use in the patient care. The spread of the next generation techniques will certainly open new perspective both in the monogenic and polygenic field.

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Chapter 8

PERIOPERATIVE ISCHEMIC STROKE

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ABSTRACT

Perioperative stroke, although uncommon, can complicate various surgical procedures leading to major consequences including mortality. In this chapter, our focus is in ischemic stroke occurring in the intra-operative and post-operative periods of non-neurological surgeries. Previously reported risk factors for perioperative stroke include a history of cerebrovascular events, chronic obstructive pulmonary disease, peripheral vascular diseases and cardiac arrhythmias. The mechanism of perioperative stroke is likely to be diverse; hence a “splitter” approach should be adopted when evaluating individual cases. Hypoperfusion is conventionally believed to be an important contributing factor in perioperative stroke. Current evidence, however, shows that its role is still controversial. Although anesthesia can induce hypotension, cerebral perfusion is seldom impaired. It is observed that watershed infarction, which is usually attributable to low blood pressure, is actually uncommon in previous case series. There are so far no good predictors for perioperative stroke. Scoring systems had been developed for stroke risk stratification, but there is no clear consensus yet in their routine applications. Various measures, including prophylactic use of statin or beta-blockers have been studied to prevent perioperative stroke.

INTRODUCTION

Perioperative stroke is an uncommon but a major complication of surgical operations with significant morbidity and mortality. Previous studies showed that perioperative stroke was a genuine clinical problem, rather than incidental events that occurred during or after

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surgery [1]. In a standard hospital, the number of surgical operations performed can be up to thousands annually. Although the incidence rate of perioperative stroke is low, the cumulative case burden is not negligible.

A substantial of patients undergoing surgery have multiple cerebrovascular risk factors, and inputs from neurologists are often needed to assess the stroke risks of surgical candidates or manage patients with perioperative stroke. Knowledge in this condition can help surgeons, anesthetists and neurologists to stratify the stroke risks in patients requiring surgery, make timely diagnosis and formulate the management plan. Moreover, by exploring the relationships among the perioperative stroke and various contributing factors, we can further understand the pathogenic mechanisms of various stroke syndromes.

In this chapter, we will limit our discussion to ischemic stroke associated with surgery, which accounts for the majority of perioperative stroke cases.

EPIDEMIOLOGY

Perioperative period is conventionally defined as the intraoperative period and a time span of 30 days after the index surgery. Stroke is defined as a focal or global neurological deficit of cerebrovascular cause that persists beyond 24 hours or is interrupted by death within 24 hours [2]. These definitions are user-friendly for diagnostic purpose. However, covert cases which are subclinical and only detectable with neuroimaging might be missed, and these patients can have resultant subtle cognitive impairment.

Currently, large scale studies to delineate the epidemiology of perioperative stroke especially in field of general surgery are lacking. The available data are mostly from relatively small or single-centered case series. The reported incidence of perioperative stroke is from less than 1% to up to around 10%, depending on the nature of intervention and whether cardiovascular procedures are included. For general surgery, the rate is less than 1%, [3] e.g., hemicolectomy 0.7%, hip replacement 0.2%, lung lobectomy or segmental resection 0.6% [4]. The incidence of perioperative stroke is about 3% for vascular surgery [5], but the risk is doubled in carotid endarterectomy [6]. The risk in head and neck surgery is 4.8% [7]. Cardiac surgery is among the highest risk groups with up to 10% risk of perioperative stroke [8, 9].

We have conducted a retrospective case-control study on perioperative stroke in our institution which is a university teaching hospital serving a population of approximate 500,000 in the southern and western districts of the Hong Kong Island. From January 2006 to July 2009, the number of non-neurosurgical operations performed was approximately 65,300 and 32 cases of perioperative stroke were identified. Each index case was matched with two control subjects in terms of age and type of surgical procedure carried. The incidence of perioperative stroke was 0.049%. This relatively low rate as compared with previous studies was probably due to the inclusion of the low risk ambulatory day cases into the analysis. Thirty-four point four percent of perioperative stroke patients underwent cardiothoracic surgery. Twenty-eight point one percent occurred in orthopedic surgery and 18.8% occurred in vascular surgery [10].

To summarize, cardiovascular procedures carry high risk because of both patient and procedural factors. Cardiopulmonary bypass carries extra embolic risk to the subject.

Vascular procedures, such as carotid endarterectomy, involve direct manipulation of the blood vessels which may result in thromboembolism to cerebral arteries. Moreover, patients with atherosclerotic vasculopathy essentially harbor various risk factors of stroke.

It is notable that most of these data were obtained from relatively small case series and were retrospective. Moreover, minor strokes with subtle clinical manifestations as well as subclinical cerebral infarcts could easily be missed and were probably not included in most of the previous studies.

Emergency procedures have been reported to be associated with a higher risk of stroke than elective operations. Patients requiring emergency operations usually have unstable systemic conditions such as sepsis, hemodynamic disturbance or organ failure [8]. The stroke onset time can range from during the intraoperative period, in which the diagnosis is usually made soon after the patients recover from general anesthesia, to two days or even weeks after the procedure (up to 30 days is the usual definition of perioperative period). The timing of perioperative stroke has been reported as bimodal. About 45% of the strokes were diagnosed within the first day after surgery and 55% were identified beyond the first postoperative day with uneventful recovery from the anesthesia [11]. Conversely, some investigators suggested most strokes occur late in the postoperative course. Hart and Hindman reported a case series of 12 patients with perioperative stroke, in whom 10 presented two days or more after the surgery [12].

PATHOGENIC MECHANISM

Hemodynamic disturbance from blood loss and hypotension induced by anesthesia during the intra- or post-operative periods could theoretically be the major culprit of stroke complication. Hence, watershed infarction from systemic blood pressure drop should have been a common perioperative stroke syndrome, and most strokes should occur intraoperatively, when the blood pressure is most liable. Nevertheless, observations from reported studies showed the contrary. Embolic stroke instead of watershed infarction was the leading cause of perioperative stroke [11, 13].

In our analysis, the majority of perioperative stroke cases (81%) were ischemic [10]. Occlusion of large arteries, probably of embolic causes, accounted for most of them, and there was only one case of watershed infarction (3%). We also analyzed the blood pressure as a stroke risk factor. Blood pressures at the time of admission and intraoperative period were studied, with equivocal results. The index cases showed greater fluctuations in some parameters only when compared with the controls. The correlation between occurrence of stroke and most of the blood pressure indices were not significant.

In a series of 61 cases of perioperative ischemic stroke after general surgery, only seven patients had watershed infarction [14]. Likosky *et al.* reported that after cardiac surgery, only 8.8% or perioperative strokes could be attributed to hypotension [15]. Embolic infarction was the commonest stroke subtype, accounting for 62.1% of all cases. Other stroke subtypes include lacunar infarction (3.1%), thrombotic stroke (1.0%), multiple etiologies (10.1%) and hemorrhagic stroke (1.0%).

Concerning the time of occurrence of embolism, researchers have monitored for the presence of embolic signals in intracranial vessels with transcranial Doppler intraoperatively

[16]. They found that the amount of embolic signals varied in different stages of cardiac surgery. The highest embolism risk occurred during the aortic cross-clamp and at the initiation of cardiopulmonary bypass. Nevertheless, the majority of the embolic strokes occur in the postoperative period remote from the surgery. Atrial fibrillation and myocardial infarction during the postoperative period are likely the main contributors to embolic stroke [14].

For non-cardiac and non-neurological operations, both thrombotic and embolic etiologies appear important. Hart and Hindman reported cardiogenic embolism was the commonest cause of perioperative stroke in general surgical procedures, accounting for 42% of all cases [12]. On the other hand, some investigators observed that the majority of perioperative stroke associated with general surgery were due to thrombosis, accounting for 68% of all cases, which was four times commoner than embolic stroke [14].

The etiology of thrombosis in perioperative stroke can be diverse. Endothelial dysfunction due to surgical stress is proposed to play a role. Unstable endothelium due to surgical stress may lead to thrombosis through various mechanisms including atherosclerotic plaque rupture and arterial spasm [17]. Besides, a hypercoagulation state is observed among surgical patients in the postoperative period. Alterations in clotting factors, platelet number and function, and fibrinolytic activity have been demonstrated. Increase in fibrinogen, activation of factor VIII, suppression of tissue plasminogen activator and decreased erythrocyte deformability all contribute to raise blood viscosity after surgery [12]. These effects can last up to weeks after the operation.

For perioperative stroke involving the vertebrobasilar circulation, there are concerns about cervical overextension leading to vertebral artery trauma [18]. In a study involving 160 patients, the effect of neck position during intubation on cerebral blood flow was investigated [19]. Magnetic Resonance Angiography was employed to monitor the blood flow changes in carotid and vertebral arterial circulation during stimulated tracheal intubation position. The results showed that posterior circulation, but not the carotid arteries, was susceptible to hyperextended neck position. This phenomenon is probably related to the anatomy of the vertebral arteries, which pass through the foramen transversarium of the transverse processes of the vertebral column. Neck angulation can result in mechanical compression on the arteries producing haemodynamical disturbance. Twenty-five percent of patients had hypoplastic vertebral arteries with low blood flow. In this patient group, basilar artery flow might be disturbed and microinfarctions were frequently found. In patients with previously unsuspected carotid and vertebral occlusion, basilar artery blood flow was also disrupted, including flow reversal, by hyperextended neck posture. Special attention to neck position should be paid for patients with known vertebral hypoplasia, vertebral occlusion or severe stenosis. A sustained neck hyperextension for more than 12 minutes seems to be a critical threshold.

Other less common pathogenic mechanisms of perioperative embolic stroke include air embolism after cardiopulmonary bypass, fat embolism after orthopedic surgery, arterial dissection and paradoxical embolism from deep vein thrombosis in patients with patent foramen ovale [11].

PROGNOSIS

Perioperative stroke is associated with poor prognosis with high mortality rate [9]. An eight-fold increase in perioperative mortality within 30 days was observed [20]. Among patients who have a history of cerebrovascular disease, mortality rates of 18% and 86% were observed [1, 14].

In our cohort, the mortality rate was 31.2% within 3 months, as compared to no post-operative death in the control group [10]. Sixty-three point three percent of perioperative stroke patients had a drop in Modified Rankin Score of 2 or greater while the same decrement was only 11% in the control group. Hence, perioperative stroke is associated with major mortality and morbidity.

Early deaths are mainly due to cerebral edema and increased intracranial pressure; late mortalities are from stroke-related complications including pneumonia, aspiration and sepsis. The high mortality may be explained by increased stroke severity for perioperative cases. The deleterious effects from ischemia tend to be accentuated by systemic inflammatory response [21]. This is precipitated post-operatively with increased levels of cytokines, including interleukin-1 and 6 and tumor necrosis factor alpha [22, 23]. The serum interleukin-6 level has been associated with brain infarct volume, 3-month clinical and stroke mortality rate outcome [24].

RISK FACTORS

Various case series had reported different risk factors for perioperative stroke. Many of them are similar to those in the non-surgical settings. We highlight some of the commonly quoted predisposing factors especially those that are specific for perioperative stroke. The risk factors can be categorised into 1) Patient, and 2) Surgical factors.

1) Patient Factors

Age and Co-morbidity

Similar to stroke in the general population, age is a non-modifiable risk factor. Studies have shown that, in patients who underwent colorectal, joint replacement and lung resection operations, perioperative stroke risk was increased in those beyond the age of 65 years old [4]. In our cohort, the median age of perioperative stroke cases was 69.9 years old [10]. Besides being an independent risk factor, age is associated with other cerebrovascular risk factors, such as hypertension, diabetes mellitus, atherosclerosis and atrial fibrillation.

Hypertension has been shown to associate with stroke in perioperative period. The incident rate of stroke was 0.9% among hypertensive patients as compared with 0.27% among normotensive patients, which represents a three- to four-fold increase in risk [25].

Vascular disease is also a risk factor for perioperative stroke. Peripheral vascular disease has been repeatedly demonstrated as a predictor of perioperative stroke (the role of cerebrovascular disease will be discussed in a later section of this chapter). In a study of post-operative ischemic stroke, the odd ratio for presence of peripheral vascular disease was 5.35

as compared with controls [14]. Peripheral vasculopathy is probably a reflection of the underlying diffuse atherosclerosis that involves the cerebral and cervical vasculatures as well.

Interestingly, chronic obstructive pulmonary disease has also been identified as a risk factor [14]. The odd ratio of perioperative ischemic stroke in patients with chronic obstructive pulmonary disease was report to be 8.77 as compared with control. One may argue obstructive lung disease is more common among elderlies and hence is associated with the risk factor of age. On the other hand, poor respiratory function has been related to increased stroke risk in the general population [26, 27]. It is speculated that hypoxemia secondary to chronic pulmonary disease may aggregate cerebral ischemia.

History of Stroke

History of stroke had been consistently demonstrated to be a risk factor in perioperative stroke. In a 10-year study conducted by Mayo clinic, Rochester, a history of previous cerebrovascular disease was shown to significantly contribute to perioperative stroke risk [14], although the time interval between patient's previous stroke and current surgery did not predict this risk. This increase of perioperative stroke risk is not surprising since this group of patients likely harbor various cerebrovascular risk factors [28]. Also, secondary prophylaxis treatments for stroke such as anti-platelet and anti-coagulation agents have to be withheld during the perioperative period. It is interesting to note that the pathogenic mechanisms of recurrent strokes in the perioperative period are often different from previous episodes. This is different from the findings in the Framingham study which suggested that recurrent stroke is likely caused by the same mechanism of previous strokes [1].

Patients with stroke or transient ischemic attack are advised not to have elective surgical procedures until up to 3 months after the initial event [1, 29]. The rationale underlying this recommendation is to allow the cerebrovascular autoregulation to recover and inflammatory response to wear off. A study has shown that the cerebral autoregulation system would be impaired after stroke not only in the affected side of cerebral hemisphere but also the unaffected side [30]. Acute stroke may impair the baroreflex and vasomotor control and hence induce a labile systemic blood pressure compromising the cerebral autoregulation. This susceptible stage may last a relative long time from days to weeks to recover. For urgent operation, blood pressure has to be carefully monitored and controlled to avoid rapid fluctuations. After the acute phase, the duration between stroke and subsequent surgical operations seems not to have any bearings on the risk of recurrent stroke during the perioperative period.

Carotid Stenosis

Carotid stenosis can be symptomatic or asymptomatic, depending on whether ipsilateral transient ischemic attack or ischemic stroke has occurred. Stroke risk stratification also depends the kind of operation patient is going to receive. Large-scale randomized clinical trial has demonstrated that carotid endarterectomy can benefit patients with symptomatic carotid stenosis of 70 to 99%, provided the operative risk is below 6% and patient's life expectancy is greater than five years [31]. Patients with asymptomatic carotid disease of 60 to 99% can also be benefited from surgery [32, 33]. However, the results of these studies may not be directly applicable to surgical candidates. These studies have commonly excluded patients with co-morbidities such as valvular heart disease, recent ischemic cardiac events, cardiac arrhythmia and organ failures which are frequently encountered among surgical patients.

It is not uncommon for clinicians to pick up asymptomatic carotid bruit as an incidental finding at pre-operative assessment. However, this clinical sign might not be reliable as only around 50% of carotid bruits in asymptomatic patients have hemodynamically significant carotid stenosis [34, 35]. Even if ultrasound screening for carotid stenosis is used, evidence for an association between the degree of stenosis and perioperative stroke is still lacking. Evans and Wijdicks have studied the risk associated with carotid stenosis in 284 patients undergoing general surgery [36]. Carotid stenosis was diagnosed by ultrasound Doppler in 224 patients, and perioperative stroke occurred in 3.6% of them. Interestingly, the degree of stenosis did not correlate with the risk of cerebrovascular complications. Although the incidence of stroke in cohort was higher than unselected populations, the investigators concluded that there was insufficient evidence to support routine carotid stenosis screening and prophylactic endarterectomy.

Another study has shown that intraoperative transcranial Doppler did not reveal significant changes in cerebral blood flow in patients with carotid stenosis [37]. The risk of perioperative stroke among coronary artery bypass graft (CABG) patients with asymptomatic moderate to severe carotid disease (defined as 50 to 99% stenosis) was 3% for unilateral and 5% for bilateral disease, compared to an overall stroke risk of 2%.

For symptomatic carotid stenosis, revascularization may benefit patients undergoing cardiac or major vascular surgery. There is still no consensus on how to manage carotid stenosis among surgical patients. Most debates are in the field of CABG. Recommended strategies include staged carotid endarterectomy before or after coronary bypass, or combined approach in which both operations are performed at the same time [38]. The carotid endarterectomy before CABG approach would increase the risk of myocardial infarction. Conversely, if CABG is performed first, the risk of perioperative stroke would be higher. The American Academy of Neurology concluded that perioperative complication rates were probably similar with combined approach when compared with carotid endarterectomy before coronary artery bypass [39].

In conclusion, there is no standard protocol in screening and managing carotid stenosis in surgical patients. The extent of preoperative investigation and indication of revascularization depends on the degree of stenosis, whether it is symptomatic or not and the nature of foregoing surgical procedure. Clinicians should take a detailed history and neurological examination, aiming to elicit symptoms of subclinical cerebrovascular events. The aim of neuroimaging studies is to look for ipsilateral silent infarct. Further investigations, including transcranial Doppler ultrasonography and computed tomography or magnetic resonance angiography, are other options to provide further information to identify patients who could benefit from prophylactic carotid revascularization.

2) Surgery Factors

Blood Pressure Control

Although hypotension does not appear to be the main cause of perioperative stroke, maintaining a stable blood pressure probably has a role in preventing surgical complications including stroke. As cerebral circulatory autoregulation has its own limitations, if the systemic blood pressure falls below the lower threshold, hypoperfusion to brain tissue occurs, leading to ischemic stroke, especially infarction in the watershed areas. Decrease in cerebral

blood flow may also accentuate ischemic injury resulted from vascular occlusion due to emboli [40]. Maintaining a higher mean blood pressure is associated with decrease in stroke risk associated with CABG [41]. However, the definition of optimal blood pressure level is still debatable. Individualized blood pressure adjustment with reference to preoperative blood pressure may be a logical approach. However, for CABG patients, this customized approach might not be better than using a predetermined arbitrary higher level of mean arterial pressure [42]. The explanation possibly lies on the diffuse atherosclerotic arterial system has reset the lower limit of the autoregulation among patients requiring coronary bypass. Maintaining a higher mean arterial pressure can in general easily overcome the lower threshold of the autoregulation. Further evidence is needed to decide whether the same measure can be applied to non-cardiac surgery.

Anesthesia

Different anesthetic agents and techniques can have different effects on the central nervous system and physiological homeostasis, especially the haemodynamics. Hence the choice of anesthesia may affect the perioperative stroke risk. Local or regional anesthesia when comparing with general anesthesia apparently associates with less systemic disturbance and hence may help to prevent perioperative stroke. In carotid endarterectomy, which both regional and general anesthesia techniques are feasible, the choice of regional anesthesia seems to lower the stroke risk. In a systemic literature review of trials comparing regional versus general anesthesia, it was found that regional anesthesia was associated with 50% reductions in the relative odds of any stroke and or death within 30 days of carotid surgery compared with general anesthesia [43]. In hip fracture repair, there is evidence to show that regional anesthesia results in less blood loss [44]. This may help to prevent stroke complication. Whether it can be generalized to other types of surgical procedure is not yet settled until further evidence is available.

Whether the hypotension induced by general anesthesia is an independent risk factor for perioperative stroke is arguable. Some investigators have found that the hypotensive stage after general anesthesia does not result in elevated stroke risk even in patients with carotid stenosis [37, 45]. The hypotensive effect may be offset by the decrease in cerebral metabolic rate and oxygen consumption due to the anesthetics. But on the other hand, cerebral blood flow to ischemic brain tissue may be reduced by general anesthesia in animal and human models. It has been described as “steal” phenomenon due to presumable vasodilation in non-ischemic areas [46]. Autoregulation impairment and hypocarbia secondary to mechanical ventilation under general anesthesia may contribute to the risk of cerebral infarction [12].

Besides the haemodynamics effect, anesthetics also have pharmacological effects on nervous tissues. The neuroprotective role of anesthetics has been studied in ischemic stroke models. For example, propofol has been shown to protect neuronal cells through various molecular cellular mechanisms [47]. Propofol also has antioxidant activity. It may attenuate neuronal injury by suppressing free radicals in reperfusion of ischemic neuronal tissues. Other anesthetics agents, such as thiopental and etomidate have neuroprotective effect by various mechanisms including decreasing the cerebral metabolic rate and oxygen consumption. Inhalational anesthetics may also play a protective role in cerebral ischemia. Short-duration halothane has been demonstrated to decrease cerebral infarction volume in an animal stroke model when compared with propofol. This protective effect did not mediate through the change in cerebral blood flow [48]. Whether these proclaimed anesthetic neuroprotective

effects which were mainly demonstrated in cell or animal models, can produce observable clinical benefit in perioperative stroke patients is a subject of further clinical studies [49].

Cardiac Surgery

Cardiac surgery can lead to various neurological complications besides stroke. A prospective study designed to study neurological complications in CABG has included 2108 patients [50]. The study found that the complications could be focal injury such as stroke or global injury such as stupor and coma or less well defined neurological outcomes such as deteriorated intellectual functions, memory deficits, or seizures. There were 129 patients, 6.1%, had adverse cerebral outcomes. These patients had relatively longer hospital stay, higher in-hospital mortality and higher rate of institutionalization.

There are issues concerning stroke after cardiac surgery that are worth discussing. First of all, the complexity of cardiac surgery is closely related to the risk of perioperative stroke. For isolated CABG the risk is up to 3.8% [8, 9]; isolated valve surgery the risk is 4.8-8.8% [8]; combined CABG and valve surgery the risk increases to 7.4% [8, 9]; for double- or triple-valve surgery the risk is 9.7% [8]. The increased risk with complexity can probably be explained by longer cardiopulmonary bypass time and operational time, more manipulation of the heart and vascular structures and greater risk of other cardiac complication such as atrial fibrillation or myocardial infarction and hence high risk of perioperative cardioembolism.

Second is that whether the off-pump surgery can reduce the perioperative stroke risk is still controversial. Extracorporeal circulatory pump system used for cardiopulmonary bypass can be a source of embolism in the pathogenesis of perioperative stroke. When blood is circulated through the system, microemboli can form over the internal surface of the extracorporeal bypass pump and its tubing, despite heparin is used [51]. Intense inflammatory response can be triggered when blood flows through the artificial system. During the bypass, the pulmonary circulation is skipped which normally functions as a filter for any microembolus flowing from the venous to the arterial system. Non-pulsatile blood flow may also favor the formation of emboli. Also, aortic instrumentation and manipulation, including cannulation, decannulation, clamping and unclamping, can result in disruption of atherosclerotic plaques in the aorta leading to cerebral embolism.

Transcranial Doppler ultrasound study has demonstrated that the off-pump operations had relatively low emboli load when compared with on-pump procedures. However, studies have shown inconclusive results in correlating off-pump surgery and stroke risk. In a meta-analysis by Wijeyesundera et al. [51], including 37 randomized control trials with 3,449 subjects, off-pump surgery showed a reduction in stroke complication but it did not reach a statistical significance (odd ratio 0.52; 95% confidence interval 0.25 to 1.05). The meta-analysis also included 22 observational studies, including 293,617 subjects. These observational studies concluded that off-pump surgery could prevent perioperative stroke (odd ratio 0.62; 95% confidence interval 0.55 to 0.69). In another large meta-analysis including 37 randomized control trials with 3,396 subjects [52], there was no statistical significant reduction in perioperative stroke with off-pump surgery (odd ratio, 0.68; 95% confidence interval, 0.33–1.40), although this study might have considerable overlapping with Wijeyesundera's study in recruiting trials for meta-analysis. Biancari et al. has reported that the off-pump coronary artery bypass surgery could prevent perioperative stroke among patients with high stroke complication risk and when intraoperative epiaortic ultrasound scanning was used [53]. The high risk group was defined by the Northern New England Cardiovascular Disease Study

Group (NNECVDSG) stroke risk score. The NNECVDSG stroke risk score is a tool for stratifying perioperative stroke risk among CABG patients and its details will be discussed further later in this chapter.

Besides developing the off-pump technique, efforts have been paid to improve the cardiopulmonary bypass system. The mini-extracorporeal circulation (MECC), is an example. It is a cardiopulmonary bypass circuit that requires a relatively small prime for the centrifugal pump. It can minimize blood-air contact as it needs neither an open venous reservoir nor direct reinfusion of cardiectomy suction from the pericardial space. It is considered to limit the systemic inflammatory response when blood flows through the system.

In a prospective randomized study, 300 patients were assigned to either MECC or conventional on-pump CABG [54]. No difference was found between the two groups in operative mortality and morbidity, transfusion rates, hospital stay, and inflammatory markers. In a smaller randomized study, 40 patients were assigned either to MECC or conventional on-pump operation [55]. The MECC was shown to have better outcomes, though the perioperative stroke risk was not specifically studied.

Third issue worth paying attention is the surgical technique in cardiac surgery. Surgery involving the manipulation of the aorta carries the risk of embolic stroke due to disruption of the aortic atherosclerotic plaques. McKhann et al. has shared the experience in reducing stroke complication of cardiac operation by adopting 'single clamp technique' [9]. Instead of the conventional 'double clamping technique' by applying two clamps, that is one aortic clamp and a side biting clamp, over the aorta, the 'single clamp technique' minimized the aorta manipulation by applying one clamp only. Use of the intra-aortic filter to capture the emboli is another feasible approach. It has been shown in prospective study that the use of aortic filter resulted in better neurological outcomes in the high risk group [56].

On the other hand, the use of epiaortic or transesophageal ultrasonography can help to identify atherosclerotic plaques in the aorta. Surgeons can decide their operational approach in view of the location and morphology of the aortic plaque [53]. Flat atherosclerotic lesions over the posterior wall of ascending aorta or at the origin of the brachiocephalic trunk are considered safe for side bite clamping as they are far from the clamping site. Unstable plaque such as the exophytic lesions are a contraindication to aortic manipulation. The ultrasonography can also assess the presence of patent foramen ovale, left ventricular or atrial thrombi which all can predispose to perioperative stroke.

PERIOPERATIVE DRUG USE

Anti-platelet Agents or Anti-coagulants

Neurologists are often consulted when to withhold the anti-platelet or anti-coagulation agents before surgery. It depends on the indication of agents and the bleeding risks of the procedure. The American College of Chest Physicians has issued guidelines on perioperative anticoagulants adjustment [57]. For patients with significant risk, such as those with prosthetic heart valves, recent cerebrovascular events and atrial fibrillation with other cerebrovascular risk factors, bridging heparin therapy is recommended while withholding oral anti-coagulation. The dosage of heparin has to be carefully administrated and the neurological

status should be closely monitored as one study has shown increased risk of perioperative hemorrhagic stroke especially in those with previous stroke [1]. On the other hand, withholding these agents may lead to rebound hypercoagulation in the postoperative prothrombotic stage.

Statins

Statins or HMG-CoA reductase inhibitors have been proposed to reduce perioperative stroke risk. Besides their lipid lowering action, these agents offer other vascular protective functions, including improvement in endothelial function, atherosclerotic plaque stabilization and decrease of inflammatory markers, the so called pleiotropic effects [58]. Statins can suppress the level of isoprenoids, which are the intermediates in cholesterol metabolism. Various cytokines and inflammatory mechanisms are attenuated by reduction in isoprenoids which plays an important role in triggering signaling pathway leadings to inflammatory cascade [58].

Surgical candidates are advised to take statins around 4 weeks before surgery, irrespective of their lipid levels. The patients with cardiovascular diseases are advised to continue the statin after the surgery as long as it is not contraindicated [58]. But there is no consensus in the drug duration in patients without cardiovascular disease. Long acting formula drugs are preferred [59].

In a large-scale meta-analysis involving over 30,000 patients, statin has been demonstrated to reduce perioperative stroke in patients undergoing cardiac surgery [60]. However, in a Cochrane review, statin use was not related to lower perioperative stroke risk in mainly cardiac revascularization recipients. The review included 11 randomized controlled studies with 984 subjects undergoing on- or off-pump cardiac operations. Statin pre-treatment before surgery reduced the incidence of post-operative atrial fibrillation, was associated with a shorter length of stay in the intensive care unit and in the hospital but failed to prevent post-operative stroke [61]. In a study on the effects of statin use on perioperative hemodynamic parameters and its clinical outcome in on-pump coronary bypass grafting or valvular surgery, the authors concluded that the treatment did not affect the hemodynamics and the surgical outcome [62]. The discrepancies in the results probably lie on the selection of studies in different meta-analyses. The controversies will likely go on until more randomized controlled trials are conducted.

Beta-Blockers

Beta-blockers were once recommended for patients preparing to have vascular surgery and patients with high risk for coronary disease preparing to have intermediate to high risk non-vascular surgery. Some studies suggested beta-blockers could prevent perioperative atrial fibrillation [63]. However, the landmark POISE trial has shown perioperative use of metoprolol was associated with a 30% decrease in non-fatal myocardial infarction but also a 117% increase in stroke [64]. In a meta-analysis included over 12, 000 patients, beta-blockers were shown to increase stroke risk, with odd ratio of around 2 [65]. The rationale was

supposed to be the induced hypotension. But since hypotension is not the main cause of perioperative stroke, there may be other mechanisms for this association yet to be discovered.

Risk Stratification

Various perioperative stroke risk-predicting models have been developed to stratify the risks among surgical candidates. McKhann et al. used three factors to predict stroke risk among patients undergoing CABG, including age, previous stroke history and hypertension. The model returns a relative probability of perioperative stroke [9]. The Northern New England Cardiovascular Disease Study Group (NNECVDSG) has proposed another model in predicting stroke risk in CABG patients [66]. The model was based on an observational study of over 33,000 consecutive CABG patients [67]. It was a scoring system consists of seven items including age, emergency of the surgery, gender of the patient, cardiac ejection fraction, serum creatinine level, presence of valvular disease and diabetes mellitus. Each item had different weighted score. Estimated stroke risk could be obtained by matching the total score from a table.

In a recent study, a model has been established trying to predict the perioperative stroke risk in non-cardiac and non-neurological surgery [20]. The model was based on a prospective study on the risk factors of perioperative stroke. It consisted of 9 identified risk factors and 1 protective factor. The risk factors included age of 62 or above; myocardial infarction within 6 months; acute renal failure; history of stroke; pre-existing dialysis; hypertension; history of transient ischemic attack; chronic obstructive pulmonary disease; and current smoking habit. High body mass index in the range of 35 to 40 kg/m² was considered as a protective factor. This contradicts to the common believe that obesity is a risk factor of cerebrovascular disease. The authors defended that higher BMI might protect against stroke-associated mortality in the elderly but not the young stroke patients. The predicting model classified the surgical candidates into 3 groups with low, medium and high risks. The odd ratios of perioperative stroke could be calculated by the statistical model used in the prospective study.

Such predictive models can help to provide feasible means for clinicians to assess the perioperative stroke risk, though the reliability have to be further confirmed. Also, whether similar models can be established for different types of operation is subjected to further studies.

Treatment

Although most cases of the perioperative ischemic stroke occur in in-patient setting, intravenous thrombolytics cannot be given due to recent surgical operation despite the diagnosis can probably be made within therapeutic windows. Intra-arterial thrombolysis is theoretically feasible option, though there is still no large scale trial to prove its efficacy and safety in perioperative stroke. In a retrospective case series, the intra-arterial thrombolytic use within 2 weeks of postoperative period achieved recanalization rate of 39% and Rankin score less than or equal to 2 at 1 week post-therapy in 38% of the patients [68]. The therapy carried 25% of bleeding risk but most were minor bleeding at surgical wound. Most of the cases with major surgical site bleeding were intracranial surgery patients. So the therapy was suggested

to be avoided in this group of patients. Whether the mechanical clot retrieval therapy can achieve the therapeutic goal with avoidance of the complication of bleeding, further studies are needed.

CONCLUSION

Perioperative stroke is an uncommon but severe surgical complication. It is associated with high mortality. Cardiac and vascular operations have the greatest risks. Embolism is a major pathogenic mechanism of perioperative stroke. Although hypotension is not a major culprit, maintaining a static blood pressure during and after the operation may probably help to prevent the complication. Anesthesia technique and anesthetics choice may modify the susceptibility or the damage due to stroke in perioperative period. Patients taking antiplatelets or anticoagulants should be managed according to bleeding risk of the procedure and the indication of the medications. Betablockers tends to increase the stroke risk due to unknown mechanism. Statins probably protect patients from stroke by the pleiotropic effects. Various investigators have developed different models to predict the perioperative stroke risk. But these are mainly in the field of cardiovascular surgery. More attention should be paid in the non-cardiac surgery. Treatment is mainly based on experience of individual institute as there is no established guideline and the expertise required may not be available in every center.

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Chapter 9

TRADITIONAL MEDICINE IN THE TREATMENT AND PREVENTION OF ISCHEMIC STROKE

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ABSTRACT

Ischemic stroke is a leading cause of death worldwide, bringing about serious long lasting disability and considerable social burden. During ischemic stroke, ischemia takes place due to blood flow interruption as a result of cerebral artery blockage and subsequently reperfusion occurs, triggering a complex cascade of molecular events that eventually results in irreversible cell death, affecting functioning of the brain and body. Sometimes vision impairment also occurs due to ischemia/reperfusion injury in the retina. Treatment of ischemic stroke is important to alleviate the subsequent outcome. Yet, no ideal neuroprotective agents are available. Some research has turned to traditional medicine, which has been used for decades. The traditional medicine has shown efficacy in cell and animal models, making it an attractive option in treatment of ischemic stroke. Another attractive alternative would be the prevention of ischemic stroke using traditional medicine, which may be beneficial for patients at high risk for ischemic stroke. This chapter aims to give an overview of the traditional medicine being tested and its efficacy in the treatment and prevention of ischemic stroke.

ABBREVIATIONS USED

Ach	acetylcholine
AMPA	2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid
BDNF	brain-derived neurotrophic factor
BNJ	Buchang Naoxintong Jiaonang
BrdU	5-bromodeoxyuridine

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BYHWD	Buyang Huanwu decoction
CAT	catalase
COX-2	cyclooxygenase-2
DJ	Danqi Piantang Jiaonang (DJ)
DPPH	1,1-diphenyl-2-picrylhydrazyl
FDA	Food and Drug Administration
GABA	γ -aminobutyric acid
GDNF	glial cell line-derived neurotrophic factor
GFAP	glial fibrillary acidic protein
GPx	glutathione peroxidase
H ₂ O ₂	hydrogen peroxide
HHD	Huang Lian Jie Du decoction
HIF-1 α	hypoxia-inducible factor-1 β
hsp70	heat shock protein 70
ICAM-1	Intercellular Adhesion Molecule 1
IL-1 β	interleukin-1 β
IL-6	interlukin-6
iNOS	inducible nitric oxide synthase
I/R	ischemia/reperfusion
KFW	Kueichih Fuling Wan
LBP	extracts of <i>L. barbarum</i> containing mostly polysaccharides
LDH	lactate dehydrogenase
LDL	low density lipoprotein
LPS	lipopolysaccharides
LTP	long term potentiation
MCAO	middle cerebral artery occlusion
MDA	malondialdehyde
MMP	mitochondrial membrane potential
MPO	myeloperoxidase
NaCN	sodium cyanide
NINDS	National Institute of Neurological Disorders and S troke
NMDA	N-methyl-D- aspartate
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
PAF	platelet-activating factor
OGD	oxygen-glucose deprivation
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
QKL	Qingkailing
QZT	Qizhu Tang
rCBF	regional cerebral blood flow
ROS	reactive oxygen species
RQKL	Refined Qing Kai Ling
rtPA	recombinant tissue plasminogen activator
SMS	Shengmai San
SOD	superoxide dismutase

TAM	Traditional African medicine
TCM	traditional Chinese medicine
TCPM	traditional Chinese patent medicine
TBARS	thiobarbituric acid reactive substances
TEM	Traditional Eastern medicine
TMP	2,3,5,6- Tetramethylpyrazine
TNF α	tumor necrosis factor α
VEGF	vascular endothelial growth factor
WHO	World Health Organization

INTRODUCTION

Stroke is a devastating cerebrovascular disease and the second leading cause of death throughout the world. Each year, more than 15 million people suffer from stroke and one-third of them die. The rest of the survivors might be left with various cruel sequelae, including hemiplegia, dysesthesia, ataxia and even visual impairment. Management of these subsequent disabilities poses an increasing burden to the whole society as the population ages. There are two major categories of stroke according to its etiology, namely ischemic stroke and hemorrhagic stroke. Of them, ischemic stroke accounts for approximately 80% of total cases [1]. During ischemic stroke, ischemia takes place due to blood flow interruption as a result of cerebral artery blockage and subsequently reperfusion occurs, triggering a complex cascade of molecular events that eventually results in irreversible cell death, affecting functioning of the brain and body. This cell death, induced by ischemia/reperfusion injury, is a complex interaction of multiple events involving apoptosis, oxidative stress and inflammation [2].

Present management for acute ischemic stroke mainly focuses on early re-vascularization including pharmacologic thrombolysis, mechanical thrombectomy and angioplasty. The key therapeutic strategy of the management is to restore the blood flow to ischemic brain tissue at an early stage in order to protect the neurons from further damage and reduce the long-term disabilities associated with stroke attack. To date, the only approved medicine for acute ischemic stroke by the Food and Drug Administration (FDA) is recombinant tissue plasminogen activator (rtPA). Approval was granted after a large, randomized, placebo-controlled study by the National Institute of Neurological Disorders and Stroke (NINDS) that showed a significant improvement in 3-month outcomes with rtPA [3]. In a 12-month follow-up study, patients with acute ischemic stroke who were treated with rtPA within 3 hours after symptom onset were at least 30% more likely to have minimal or no disability than placebo-treated patients at 12 months [4]. Despite the wide acceptance of rtPA in ischemic stroke treatment, there still remains controversy since it increases the risk of symptomatic intracranial hemorrhage [3-5]. In this case, rtPA is only recommended for carefully selected patients who are eligible according to the treatment criteria. The restrictive 3-hour time window necessary for the use of rtPA further limits the number of patients who will receive the intervention. It is estimated that only about 1-2% of patients with acute ischemic stroke have received this intervention [6].

Due to the narrow time window and adverse effects after ischemic stroke such as hemorrhagic transformation, ischemia/reperfusion damage and re-occlusion after recanalization, neuroscientists have become more emphasized on neuroprotection in the treatment of ischemic stroke. Physicians and neurologists have attempted to approach stroke patients with combining neuroprotective agents to salvage cell death. Currently the most studied neuroprotective agents include glutamate receptor antagonists, ion channel modulators, anti-inflammatory agents, and free radical scavengers [7]; simply speaking, in almost all components of the ischemia cascade. Despite the determined efforts in basic research and clinical investigation, neuroprotective therapies for acute ischemic stroke remain unsuccessful. They all appear to be effective in initial studies using a variety of animal stroke models; however, the outcomes of the subsequent clinical trials are far less from satisfactory. None of them have been proven effective in clinical practice [5].

With an increasing population of elderly people worldwide, stroke has become a major healthcare problem. Although the incidence of stroke has been reduced by preventive measures such as controlling hypertension, hypercholesterolemia and smoking, there remains an urgent need for effective and widely applicable pharmacological treatment. This lack of treatment for stroke may explain the growing attention in traditional medicine in the last few decades, in which abundant clinical data as well as extensive observational and anecdotal experiences have accumulated over the past millenniums.

Chinese herbal medicine has been used to treat ischemic stroke for ages. Although its efficacy has been corroborated by the outcomes of patients, the underlying mechanisms and specific active ingredients still remains unknown. Dozens of Chinese herbs, either crude or extract, have been tested in various pre-clinical animal studies and clinical trials.

This article reviews their distinct abilities in both prevention and treatment of ischemic stroke.

TRADITIONAL MEDICINE – AN OVERVIEW

In 2003, the World Health Organization (WHO) defined traditional medicine as “health practices, approaches, knowledge and beliefs incorporating plant-, animal- and mineral-based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being” [8].

Traditional medicine, also termed as complementary or alternative medicine, arises in ancient times before the establishment of modern Western medicine and is developed in various regions around the world over generations. Traditional African medicine (TAM) and Traditional Eastern medicine (TEM) are the two major branches. Indeed, traditional medicine has been used in African and Asian communities for thousands of years, and shows increasing popularity in Western society. According to WHO’s statistics and investigations, there are approximately 80% of the population who depends on traditional medicine for primary healthcare in some African and Asian countries, while in many developed regions, such as Europe and North America, 70% to 80% of the population has at least once being treated by traditional medicine [9].

Traditional African Medicine (TAM)

The pivotal theoretical concept of TAM relies on the belief in the supernatural [10], in which serious illness can be underpinned e.g., by ancestor spirit anger [11, 12]. TAM practitioners usually use divination as a diagnostic process [13] and treat the disease with ritualized use of plant- or animal-derived preparations. Plant remedies are commonly used for dealing with minor illnesses such as fever, aches and pains [14, 15] without recourse to spiritual evocations in most African communities. For more persistent illnesses or when the illness becomes life-threatening, a specialist is consulted and divination is used.

Studies have now revealed that there are many useful molecules in the plant preparations with anti-inflammatory properties. These include salicin from willow (*Salix alba*), quinine from cinchona (*Cinchona succirubra*), artemisin from quin hao (*Artemisia annua*), and gedunin from *Azadirachta indica* [15-17]. However, there seemed to be scarce reported investigations about plant remedies in TAM in treating stroke. In addition, the lack of scientific explanation of TAM has restricted its intensive study and development.

Traditional Chinese Medicine (TCM)

Apart from the African society, traditional medicine has been mostly accepted in the eastern countries, such as China, Japan and Korea. The use of traditional medicine in China, also known as traditional Chinese medicine (TCM), is very prevalent due to not only its long history but also its recognized efficacy. TCM is a precious heritage of China. Its usage, safety and effectiveness have accumulated over thousands of years and are verified by the long-term practice in the Chinese population. Its practical value is unquestionable; yet, its success would only be more convincing if the effectiveness can be explained by modern scientific theories and methodologies. The vast experience with TCM in human provides a strong basis for modern basic pharmacological research.

STROKE

Cerebral Ischemia – Pathophysiology and Mechanisms

The pathophysiology of cerebral ischemia has been well covered by many stroke researchers [2, 18]. In ischemic stroke, complex spatial and temporal events evolve over hours or even days.

Cerebral ischemia occurs as a result of an obstruction in one of the major cerebral blood vessels, resulting in reduced blood supply and therefore insufficient glucose and oxygen delivery to affected areas in the brain. An ischemic core and the penumbra surrounding it are developed [19, 20]. As appropriate blood supply is essential to maintain proper neuronal function in the nervous system, neuronal activity declines rapidly when the blood flow drops below 25% of normal values. Within the core of the ischemic territory where blood flow is most severely restricted, cellular homeostasis could not be supported and severe impairment of ATP-dependent cellular activities occurs, leading to excitotoxic and necrotic cell death [21,

22]. Not only are the neurons affected, other cells such as glia and endothelial cells are also damaged.

Ischemic stroke triggers a complex cascade of cellular and molecular events associated with ischemia/reperfusion (I/R) injury. The cascade begins with energy depletion, disruption of ion homeostasis, depolarization, calcium channel dysfunction, excessive glutamate release, excitotoxicity, and release of free radical that rapidly result in cell death in the infarct core. These early events are followed by late events such as inflammatory changes, apoptosis and disruption of the blood-brain barrier [23].

Yin-Yang Theory in TCM

The underlying principles of TCM are very different from Western medicine. Theoretically, various doctrines such as ‘Yin-Yang’, ‘Five Elements’, five ‘Zang’ and six ‘Fu’ viscera, and ‘Meridians’ (channels and collaterals) are involved [24]. In the Yin-Yang theory, ‘Yin’ and ‘Yang’ are two opposing but complementary forces of energy. They govern the essential organs in our human body, especially heart, lungs, liver, kidneys, and spleen. It is believed that diseases originate from the imbalance between ‘Yin’ and ‘Yang’. For TCM practitioners, treatment is based on this theory and they use different approaches to restore the balance between ‘Yin’ and ‘Yang’.

Basic Principles of Stroke in TCM

In TCM, stroke is a syndrome known as ‘wind stroke’. It is characterized by a sudden appearance of facial paralysis, impeded speech and/or hemiplegia. Five pathogenic factors are involved: ‘external wind’, ‘internal liver wind’, ‘phlegm’, ‘fire’, and ‘stagnation’.

These five factors can interact with each other, which, in turn, may lead to ‘Yin’ or ‘Qi’ (internal strength) weakness, ‘liver fire’, ‘wind phlegm’, ‘phlegm dampness’, or ‘blood stasis’ [25]. This results in imbalance between Yin and Yang, disturbance of the blood and ‘Qi’ circulation, deficiency of ‘liver-Yin’ and ‘kidney-Yin’, stagnation of phlegm and dampness, and eventually sudden onset of wind stroke [25].

TRADITIONAL CHINESE MEDICINE AND STROKE THERAPY

In TCM, the treatment of stroke aims to restore the equilibrium of the relative strength between the patient’s body resistance and the intensity of endogenous and exogenous pathogenic factors [25, 26]. According to the cause of wind stroke, different TCM treatments are given. These include heat-clearing drugs, anti-rheumatics, drugs for promoting blood circulation, drugs for dispersing external wind, drugs for relieving phlegm, drugs for subduing interior wind, drugs for resuscitation, and tonics for deficiency syndromes [25, 27].

Some of the actions of TCM in stroke therapy can be explained based on the pathophysiology of cerebral ischemia. They contain agents with anti-oxidative, anti-inflammatory and anti-thrombotic properties. For example, many herbs contain flavonoids,

whose anti-oxidative activity has been shown *in vitro* [28]. Flavonoids are also shown to have anti-inflammatory activities [29, 30], which may be desirable in stroke treatment. In addition, some herbs can dilate blood vessels and suppress platelet aggregation [31].

Herbal medicine, prescriptions, acupuncture, massage, and dietary therapy constitute the various forms of TCM treatment. Among them all, the usage of herbs, prescriptions and acupuncture are intensively investigated in the prevention and treatment of stroke.

Herbs Used for Treatment or Prevention of Stroke in TCM

There are more than 100 herbs that have been used in stroke prevention or treatment. Some of their active ingredients have been described in detail [25, 27, 32]. Examples of the more commonly used herbs are discussed below in alphabetical order. The active components in these herbs and their mechanisms of action in relation to cerebral ischemia pathophysiology stroke are also described.

Acanthopanax Senticosus Harms

The stem bark of *Acanthopanax senticosus* Harms (*Eleutherococcus senticosus* Maxim, *Araliaceae*) is very often called Siberian Ginseng. It is used to strengthen 'Qi' in TCM [33]. It has also been used as a tonic for chronic bronchitis, hypertension, ischemic heart disease, and anti-stress [34]. Siberian Ginseng exhibits effects such as anti-fatigue, anti-stress, immuno-enhancing, and anti-depressive effect [35]. It is also shown to be hepato-protective through its antioxidant activities [36, 37].

From the stem bark, many phenolic compounds including lignan glycosides (the glycosides of pinoresinol, medioresinol and syringaresinol), isofraxidin, isofraxidin 7-O-glucoside, chlorogenic acid, 2,6-dimethoxy-p-benzoquinone and syringin have been isolated [38]. Another component, liriodendrin, is also isolated and has been demonstrated to have anti-inflammatory effects. It significantly inhibits the increase of vascular permeability induced by acetic acid in mice and reduces the acute paw edema induced by carrageenan in rats [34]. On the other hand, the saponins isolated from the leaves can reduce the size of acute myocardial infarcts in dogs [39].

Angelica Sinensis, A. Gigas

Angelica sinensis is used in China while *Angelica gigas* is used in Korea [27]. The dried root of *A. sinensis*, also known as *Radix angelica sinensis*, is commonly used as a treatment regimen for anemia or some circulatory disorders [40]. It has also been used to treat menstruation and menopause syndrome in women [27].

A. sinensis root extracts ameliorate scopolamine- and cycloheximide-induced amnesia, but not p-chloroamphetamine-induced amnesia in rats [41]. *Radix angelica sinensis* is shown to display free radical scavenging properties [42]. Moreover, *Radix angelica sinensis* can dose- and time-dependently protect human umbilical vein endothelial cells (ECV304) against hydrogen peroxide damage and suppress the production of reactive oxygen species (ROS) [43].

Several active components have been identified in *Radix angelica sinensis*. They are ferulic acid, ligustilide, angelicide, brefeldin A, butylidenephthalide, butyphthalide, succinic

acid, nicotinic acid, uracil, and adenine [27]. Among them, ferulic acid has demonstrated neuroprotective properties. Ferulic acid significantly reduces cerebral infarct and neurological deficit-score in rats after middle cerebral artery occlusion (MCAO), may be by suppressing superoxide radicals in the parenchyma lesion and intercellular adhesion molecule 1 (ICAM-1) immunoreactive vessels in the ischemic striatum as well as reducing the expression of ICAM-1 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) in the ischemic cortex and striatum [44].

The crude extract of *A. gigas* roots also displayed anti-nociceptive properties in various pain models [40]. Long-term administration of extract of *A. gigas* root or decursinol exerts protection against β -amyloid peptide-induced memory impairment in mice [45]. Decursin, one component in the root of *Angelica gigas*, mitigates amnesia induced by scopolamine in mice [46].

Astragalus Membranaceus

Astragalus membranaceus is a herbal medicine routinely used in patients with stroke or chronic debilitating diseases in China for decades. Even normal subjects who wish to further improve their vital functions regularly use *A. membranaceus* [47]. A previous pilot study suggested that *A. membranaceus* is safe and may be beneficial in treating acute cerebral infarction [48].

Astragaloside IV is a saponin and the major active constituent of *Astragalus membranaceus*. Previous pharmacological studies showed that Astragaloside IV displays a series of protective effects; these include anti-hypertension [49], anti-inflammation [50], anti-infarction [47], anti-nociception [51], anti-viral activity [52], and positive inotropic action [53]. *In vitro* studies have shown that Astragaloside IV is a strong scavenger for superoxide radicals and hydroxyl radicals [54]. *In vivo* studies also showed that Astragaloside IV is neuroprotective in a number of ischemia models. It reduces infarct volume as well as decreases the levels of malondialdehyde (MDA) and increases the levels of antioxidant enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD) in ischemic mouse tissue after MCAO-induced I/R injury [47]. In addition, Astragaloside IV protects the blood-brain barrier and reverses the decreased expression of tight junction proteins in a rat model of focal I/R injury [55]. It was further shown in the same model that Astragaloside IV can prevent neutrophil accumulation and suppress neutrophil adhesion-related molecules, thereby exerting neuroprotection against I/R injury [56]. Moreover, Astragaloside IV pre-treatment is shown to be neuroprotective and these effects may be associated with improved cellular energy metabolism, inhibition of JNK pathway activation and then suppression of the mitochondrial apoptosis pathway [57].

Bombycis Corpus

Bombycis corpus is the dried larva of the silk moth *Bombyx mori*, dead as a result of the infection by the fungus *B. bassiana* [27]. It is used to treat symptoms induced by stroke such as convulsion, headache, palsy, and speech problems. *B. corpus* is protective against cytotoxicity induced by A β ₂₅₋₃₅ in cultured astrocytes through the inhibition of lipid peroxidation and protection of anti-oxidative enzymes [58]. Sphingolipids isolated from *B. Corpus* have demonstrated neurotrophic effects in PC12 cell neurite outgrowth [59]. Pre-treatment of *B. corpus* not only reduce the N-methyl-D- aspartate (NMDA)-induced

neurotoxic actions in the hippocampus but also protected cultured neurons against the neurotoxic actions of either 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) or kainic acid [60]. Because the release of glutamate has been implicated in cerebral ischemia injury, *B. corpus* may contribute significantly to protect the human brain.

Carthamus Tinctorius

Carthamus tinctorius (Honghua in Chinese) is the flower (or petal) of the Chinese safflower. This material is used in TCM for a wide variety of disorders, but most often for conditions expected to benefit from increased local or visceral blood flow. These include topical application for traumatic wound healing and systemic administration for amenorrhea, coronary heart disease and cerebrovascular disease [61]. In the *ex vivo* chick embryo retina assay, Honghua protects against excitotoxicity caused by glutamate, NMDA, kainate, and quisqualate as well as against neuronal degeneration caused by simulated ischemia. In the *in vivo* adult rat retina, intravitreal injection of Honghua greatly reduces ischemic damage [61]. In rats challenged with MCAO, Honghua extracts exhibit significant neuroprotective effects in decreasing neurological deficit scores and reducing the infarct size [62]. *In vitro* studies in cultured cortical neurons show that Honghua extracts significantly inhibit neuron damage induced by exposure to glutamate and sodium cyanide (NaCN) [62].

Corydalis Yanhusuo

In TCM, the tubers of *Corydalis yanhusuo* are used mainly as an analgesic in the treatment of gastric and duodenal ulcer, rheumatism and dysmenorrhea [27]. The tubers contain pharmacologically active alkaloids that have analgesic [63], anti-inflammatory [64], anti-thrombotic [65], and anti-hypertensive effects [66]. Several active ingredients have been identified. One of them is DL-tetrahydropalmatine, whose pre-treatment provides neuroprotective effect in rats affected by heatstroke [67]. It also protects neurons [68], improves neurological status and reduces the cerebral infarct size [69] in rats after I/R injury. Protopine is another component that has an inhibitory activity on platelet aggregation in rabbits [70].

Ginkgo Biloba

Ginkgo biloba L. is a tree indigenous to China [71]. *Ginkgo biloba* L., its leaves in particular, has a long history of being used for medicinal purposes [72, 73]. *G. biloba* extract has been widely used to treat circulation disorders [74], cerebrovascular insufficiency [75], peripheral vascular disease such as arterial occlusive diseases [76], age-dependent damages [77] and dementia [78]. The mechanism and clinical indications of *G. biloba* extract have been well covered by Diamond et al. [77].

The primary compounds of interest in ginkgo are ginkgolides and flavonoids, which exhibit bioactivity. In a recent HPLC analysis, the quality of *G. biloba* extract has been standardized as containing 22 – 27% w/w flavonoid glycosides and 6% w/w terpenoids [73]. EGb 761 is a standardized extract of *G. biloba* leaves. It contains ~24% flavonoid glycosides (primarily quercetin, rutin, kaempferol and isohamnetin), 6% terpene lactones, 2.8-3.4% ginkgolides A, B and C, and 2.6-3.2% bilobalide [27]. The flavonoids in EGb appear to possess strong free radical scavenging and anti-inflammatory properties [79, 80] while the

terpenoids and ginkgolides A and B inhibit platelet-activating factor (PAF) and decrease free radical release [81, 82].

Treatment with *G. biloba* extract has shown beneficial effects in reducing reperfusion injury in global ischemia and trauma experimental models [83]. *G. biloba* extract reduces infarction volume in mice with MCAO and reperfusion [84, 85]. In particular, EGb 761 reduced the size of cerebral infarction and improved neurological behavior in rats with transient and permanent focal cerebral ischemia, partially attributed to the beneficial effects of selectively improved local cortical blood flow in the area at risk of infarction [86].

G. biloba extract has both a scavenging effect on superoxide anions and an enhancing effect on SOD activities, which prevents oxidative stress in neurons [87, 88]. Free radical scavenging activity was also previously demonstrated in *G. biloba* L. leaves, which is likely to be attributed to the presence of quercetin and rutin that have free radical scavenging activity [89]. EGb 761 also shows anti-oxidative activities [90].

In particular, bilobalide, a constituent of *G. biloba*, demonstrates potent neuroprotective effects. Bilobalide pre-treatment reduces the cerebral infarct size [91]. It also suppresses hypoxia-induced membrane breakdown in the brain [92] and inhibits NMDA-induced phospholipase A2 activation and phospholipid breakdown in rat hippocampal slices [93]. Bilobalide can induce the expression of glial cell line-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF) in rat astrocytes *in vitro* [94].

Despite the evidence and similar to other TCM herbs, many randomized controlled trials for *G. biloba* extract were of inferior quality [95]. One placebo-controlled trial was assessed to be of good quality, but it failed to show an improvement of neurological deficit at the end of treatment using *G. biloba* extract [95]. There is no convincing evidence to demonstrate the effectiveness of the routine use of *G. biloba* in promoting recovery after stroke. Large-scale randomized controlled trials of high quality are necessary [96].

***Ligusticum Wallichii* Franchat**

Ligusticum wallichii Franchat (Chuan Xiong) has been used for at least 200 years by TCM practitioners [97.] It is commonly used to stimulate blood circulation and relieve pain [97] as well as to treat neurovascular and cardiovascular diseases [98]. 2,3,5,6-Tetramethylpyrazine (TMP) is one of the most important active ingredients isolated from Chuan Xiong [99]. TMP pre-treatment is neuroprotective through the suppression of inflammatory reaction and reduction of neuronal apoptosis in focal cerebral ischemic/reperfusion injury [100]. In addition, TMP protects the gerbil hippocampus against global cerebral ischemia [101] and rat brain against MCAO-induced focal cerebral ischemia [102]. Moreover, in rats with transient focal cerebral I/R injury, administration of TMP within a 4-hour time period post-stroke may reduce cerebral ischemic reperfusion damage [103]. In PC12 cells exposed to oxygen-glucose deprivation (OGD), TMP increases the survival rate of PC12 cells activation of the ActA/Smad signaling pathway and its anti-oxidative activities [104].

Two clinical trials were previously performed to test the efficacy of Chuan Xiong. However, the quality of these trials was low and did not yield any reliable evidence [105]. Similar to other TCM herbs, large-scale randomized controlled trials of good quality are necessary for any clinical recommendation.

Lycium Barbaarum L.

The fruit of *Lycium barbarum* (also named Wolfberry, *Fructus Lycii*, Gouqizi) has been widely used in Chinese herbal recipes for thousands of years. According to traditional Chinese medicine literature, *L. barbarum* can nourish the liver and kidney, helping the re-balance of 'Yin' and 'Yan' in the body. *L. barbarum* is an important ingredient in traditional Chinese medicine in promoting health and longevity. It is also taken as a food supplement in the Western countries.

L. barbarum has an attractive red color, leading to the belief that it has a role in strengthening eyesight and protecting the eyes. Chemical analysis of *L. barbarum* shows that it indeed contains lots of carotene and zeaxanthin, which can provide nutrients and antioxidants directly to the eyes [106]. Yet, carotene is normally not obtained from *L. barbarum* in our daily diet. In *L. barbarum*, the polysaccharides constitute more than 40% of the fruit extract [107]. Numerous experimental studies have revealed that *L. barbarum* has a wide array of functions, which may be due to its high polysaccharide content. These include anti-aging, anti-tumor, cytoprotective, neuromodulation, and immune modulation effects [107]. In addition, pre-treatment using extracts of *L. barbarum* containing mostly polysaccharides (LBP) can protect the cultured primary cortical neurons from β -amyloid peptide neurotoxicity [108, 109]. We also found that LBP pre-treatment could effectively protect the retina from neuronal death, glial activation and oxidative stress in a murine retinal I/R model [110]. More importantly, oral LBP pre-treatment effectively improves neurological deficits, decreased infarct size and cerebral edema after MCAO-induced I/R injury. BBB disruption, aquaporin-4 upregulation and glial activation are attenuated by LBP pre-treatment [111]. These results suggest that LBP may be used as a prophylactic neuroprotectant in patients at high risk for ischemic stroke.

Magnolia Officinalis Rehder Et Wilson

Magnoliae Cortex is the dried bark of *Magnolia officinalis* Rehder et Wilson (China) and *Magnolia obovata* Thunberg (Japan) [112]. It is commonly prescribed in TCM for the relief of stroke, myocardial infarction, headache, anxiety, diarrhea and fever [113]. Many pharmacological activities of extracts from Magnoliae Cortex have been reported; these include muscle relaxation [114], central depressant effect [115], vasorelaxant [116], anti-allergic [117], anti-bacterial [118, 119], and neurite spouting activities [120].

Two active components, magnolol and its isomer, honokiol, have been isolated. Magnolol has been found to have anti-platelet effect [121], vasodilatation effect [116] and free radical scavenging activities [122]. In the heart, magnolol may protect the myocardium against ischemic injury and suppress ventricular arrhythmia during ischemia and reperfusion [113]. Magnolol also significantly attenuates the increase in striatal ischemia and damage markers associated with heatstroke [123]. Honokiol is also reported to have hydroxyl radical-scavenging [124] and anti-platelet effects [125]. It also inhibits the production of nitric oxide (NO) and TNF α in lipopolysaccharides (LPS)-activated macrophages [126]. In addition, it protects the rat heart and liver mitochondria against lipid peroxidation [122, 127]. Further studies show that honokiol is able to reduce infarct size and exhibit an anti-arrhythmic effect in rats subjected to coronary artery occlusion [113, 128, 129]. Moreover, both honokiol pre-treatment and treatment significantly reduce the infarction volume in rats subjected to focal cerebral ischemia [130].

***Paeonia Suffruticosa* Andrews, *Paeonia Lactiflora* Pall**

Both *Moutan cortex* of *Paeonia suffruticosa* Andrews and the root of *Paeonia lactiflora* Pall are common TCM herbs for treating inflammatory and pyretic disorders [131-133]. In particular, the root bark of *P. suffruticosa* Andrews is prescribed for treating blood stagnation [27]. It demonstrates strong superoxide- and hydroxyl radical-scavenging activity [134]. It also strongly enhances cell viability against hydrogen peroxide (H_2O_2)-induced oxidative damage, together with high levels of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and enhanced activities of SOD, catalase (CAT) and GPx [135]. α -benzoyloxypaeniflorin is an antioxidant monoterpene glycoside isolated from *P. suffruticosa* Andrews. It also exhibits radical scavenging activity on DPPH radical [136].

The root of *P. lactiflora* Pall is commonly used in TCM to treat abdominal pain and blood deficiencies [27]. Previous studies showed that its extract and paeoniflorin, a major constituent of *P. lactiflora* Pall root, attenuate scopolamine-induced cognitive impairment [137]. Pre-treatment with paeoniflorin also significantly prevents the scopolamine-induced decrease in acetylcholine (ACh) content in the striatum [137]. Paeoniflorin ameliorates memory disruption mediated by adenosine A1 receptors, implicating its beneficial effect on learning and memory impairment in rodents by modulation of adenosine-mediated inhibition of long term potentiation (LTP) in the hippocampus [138]. Paeoniflorin post-treatment not only significantly reduces the infarct volume but also ameliorates the deficits in neurological symptoms caused by transient MCAO at the chronic stage [139]. Paeoniflorin is also shown to confer neuroprotection by activating adenosine A1 receptor in rat after transient MCAO [140]. Later studies show that both pre-treatment and post-treatment with paeoniflorin reduce the ratio of cerebral infarction area [141]. In particular, pre-treatment with paeoniflorin reduces the neurological deficit score together with an inhibition on the presence of ED1, interleukin-1 β (IL-1 β), tumor necrosis factor α (TNF α), ICAM-1 in microvessels and myeloperoxidase (MPO) immunoreactive cells, suggesting an anti-inflammatory effect of paeoniflorin [141]. Paeonol is a common component in the *Moutan cortex* of *P. suffruticosa* Andrews and the root of *P. lactiflora* Pall. It has been shown to be neuroprotective in ischemia/reperfusion injury. It displays antioxidant activities [142] and inhibits the aggregation of platelets in rabbits [131]. Paeonol is shown to protect cerebral ischemia by inhibiting accumulation of Ca^{2+} and production of oxygen free radical [143]. In cultured rat hippocampal neurons exposed to OGD, paeonol treatment increases cell survival rate and reverses the of intracellular Ca^{2+} overload [144]. Further investigations show that paeonol pre-treatment and post-treatment reduce the cerebral infarction area while paeonol pretreatment also reduces the neurological deficit, suggesting its potential in treating stroke patients [145]. Paeonol can scavenge superoxide anion as well as inhibit microglia activation and IL-1 β in I/R injured rats [145]. Post-treatment with paeonol reduces inflammatory responses in cultured LPS-activated microglia and oxidative stress-induced neurotoxicity in rat primary cortical neurons [146]. Paeonol also protects memory in rats after MCAO via reducing levels of amyloid precursor protein and β -secretase as well as apoptosis [147].

Panax Ginseng

Ginseng, the root of *Panax ginseng*, is a famous TCM. It is only used at least 4-6 years after cultivation in a variety of prescriptions. Numerous studies have demonstrated the usage of ginseng for 'Qi' invigorating and cardiovascular effects [27].

P. ginseng extracts decrease lipid peroxides and increase the expression of GPx and SOD, thereby protect against global ischemia injury in rat hippocampus [148]. Pre-administration of *P. ginseng* extracts protects against transient global cerebral ischemia in the rat [149] and gerbil [150].

Ginsenosides are the main active ingredients in *Panax ginseng* [27]. One of them, ginsenoside Rd, has demonstrated neuroprotective effects. Ginsenoside Rd rescues cultured hippocampal neurons that are exposed to OGD [151]. Besides attenuating apoptotic neuronal death, ginsenoside Rd also reduces the intracellular reactive oxygen species and MDA production, increases glutathione content as well as enhances the antioxidant enzymatic activities of CAT, SOD and GPx. Ginsenoside Rd can also stabilize the mitochondrial membrane potential [151]. Another ginsenoside, ginsenoside Rb1, also displays neuroprotective effects. Ginsenoside Rb1 protects the rat from focal cerebral I/R injury [152]. When given after forebrain ischemia, ginsenoside Rb1 protects hippocampal CA1 neurons against lethal ischemic damage possibly by scavenging free radicals [153]. In non-human primates, ginsenoside Rb1 has been shown to ameliorate both early and delayed injuries in the thromboembolic stroke model [154]. Interestingly, ginsenoside Rg1, unlike ginsenoside Rb1, has no effects in cerebral I/R injury [152]. Yet, ginsenoside Rg1 can increase ischemia-induced cell proliferation and survival in the dentate gyrus of adult gerbils after transient global ischemia [155].

P. notoginseng Burk is another herb from the same genus as *P. ginseng*. Its extracts have also demonstrated neuroprotective effects. *P. notoginseng* extracts attenuate the infarct size in ischemia rat brain, together with reduction in microglial activation and inhibition on production of inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 [156]. In particular, the saponins found in *Panax notoginseng* also demonstrate protective effects in cerebral ischemia [157, 158]. *Panax notoginseng* Burk can attenuate the reduction in learning and memory functions in rats with chronic stage ischemia-reperfusion injury, possibly by increasing the amount of activated microglia and brain-derived neurotrophic factor (BDNF) [159].

Pueraria Lobata

The dried roots of *Pueraria lobata* (Wild.) Ohwi have been widely used in TCM for centuries. The extracts are shown to have positive effects on I/R in the heart [160] and arterial obstruction in the retina [161]. Three major isoflavonoid compounds have been isolated from the extracts: puerarin, daidzin and daidzein [27]. Isoflavonoids in *P. lobata* have been shown to increase the cerebral blood flow in mice [162] and display anti-oxidative activities [163].

In vitro studies show that puerarin protects cultured mouse cerebral cortical neurons against glutamate excitotoxicity [164] and cultured rat cerebral cortical astrocytes against OGD [165]. The neuroprotective effect of puerarin on cultured hippocampal neurons after OGD is further shown to be associated with inhibition on glutaminergic transmitter, intracellular Ca²⁺ elevation and neuronal nitric oxide (NO) synthesis [166].

Puerarin can increase cerebral blood flow in dogs and attenuate cerebral or spinal ischemia/reperfusion injury in rats or rabbits [167, 168]. In rats subjected to MCAO, puerarin reduces the infarct volume and improves neurological function together with decreased caspase-3 activity, suggesting an inhibition of apoptosis [169]. The neuroprotective effect of puerarin is further linked with inhibition of excitatory amino acid efflux in rat after MCAO [170]. Moreover, the neuroprotective effect of puerarin is suggested to be mediated by

inhibition of hypoxia-inducible factor-1 α (HIF-1 α and TNF α , followed by the inhibition of inflammatory responses, apoptosis and neutrophil activation [171]. Recent investigations show that puerarin can reduce cerebral ischemic volume, relieve Ca²⁺ overload [172] and decrease the expression of NMDA receptor [173] while the time window for puerarin administration may be 12 hours after ischemic injury [172]. In a randomized controlled trial, the flavonoids of puerarin are shown to limit the increase of serum interleukin-6 (IL-6) and enhance blood flow in the ischemic region [174].

Based on the *in vitro* studies and animal experiments, puerarin has shown effects that may be beneficial in the treatment of acute ischemic stroke. Previously clinical trials have been conducted to assess the efficacy and safety of puerarin. However, only one trial with a small number of patients (up to 2008) can be included according to the present standard of clinical research and this trial cannot provide evidence for recommendation of routine puerarin use in all patients with acute ischemic stroke [175].

Rhodiola Rosea L., Rhodiola Sacra S. H. FU, Rhodiola Sachalinensis A. BOR

Rhodiola Radix is a Tibetan folk medicine prepared from several alpine *Rhodiola* plants and is used as a hemostatic, tonic and contusion [176]. It has been reported that *Rhodiola* plants improve learning and memory [177].

Salidroside is one of the most potent compounds in *R. rosea* L. [178] and has long been used as an adaptogen in TCM. Diverse pharmacological activities have been reported for salidroside, including anti-oxidation [179], anti-aging [180], anti-fatigue [181], and anti-carcinogenic [182]. Potent neuroprotective activity has also been described for salidroside recently. Salidroside exhibits protective effects against e.g., hydrogen peroxide-induced apoptosis in SH-SY5Y cells [183], hypoglycemia and serum limitation induced cell apoptosis in cultured PC12 cells [184], hypoxia-induced abnormal processing of amyloid precursor protein [185], and β -amyloid-induced oxidative stress in SH-SY5Y [186].

The extracts from 2 related plants, *R. sacra* S. H. FU and *R. sachalinensis* A. BOR, have also been tested in previous studies. Several components are shown to exert protective effects against cell death induced by either β -amyloid, staurosporine or H₂O₂ [187]. Phenolic compounds isolated from the roots of *Rhodiola sachalinensis* also exhibit significant scavenging effects against DPPH free radicals [188]. Moreover, *Rhodiola sachalinensis* extracts show neuroprotective effects on transient focal cerebral ischemia in rats [189].

Salvia Miltiorrhiza Bunge

The dried root of *Salvia miltiorrhiza* Bunge is known as *Radix salviae miltiorrhizae* (Danshen, also known as Tanshen in Japanese and some older literature) [190]. It is red in color and is commonly used in treatment of disorders related to blood stasis [27]. According to TCM theory, cerebral infarction is a result of blood stasis; therefore, one treatment of cerebral infarct is to quicken the blood and disspell the stasis [191]. Clinical trials indicated that Danshen was an effective medicine for angina pectoris, myocardial infarction and stroke [192].

In the United States and Europe, Danshen products can be purchased from herbal shops. In China, Danshen is available in various pharmaceutical forms, including tablets, capsules, granules, injectables, oral liquids, sprays, and dripping pills [193-196]. These various forms contain either Danshen alone or a composite of *Salvia miltiorrhiza*, *Panax notoginseng*, and

Cinnamomum camphora (known as Fufang Danshen) [193-195]. Among them, the two most commonly used forms are the Fufang Danshen Tablet and Fufang Danshen Dripping Pill. They have been officially listed in the Chinese pharmacopoeia [197]. Moreover, the Fufang Danshen Dripping Pill was the first TCM product approved for phase II and phase III clinical trials by the Food and Drug Administration (FDA) in 1997 (IND No.56956) [198].

The beneficial effects of Danshen on ischemia have been demonstrated. Danshen in experimental studies dilates coronary arteries, increases coronary blood flow, and scavenges free radicals in ischemic diseases [192]. In cerebral ischemia, Danshen attenuates the dysfunction of substance P [199] and improves ischemic cell changes by modulating somatostatin metabolism [200]. In addition, Danshen significantly decreases the stroke index by inhibiting the pre-synaptic releasing of glutamate and stimulating the releasing of γ -aminobutyric acid (GABA) in gerbils after common carotid artery ligation [201]. In a 4-vessel occlusion and reperfusion rat model, Danshen increases the cerebral SOD activity while decreasing the cerebral MDA level, suggesting that Danshen can reduce the lipid peroxidation and provide protection against cerebral reperfusion injury [202]. Further studies show that Danshen reduces the cerebral NO concentration to normal levels [203]. Both Danshen pre-treatment [204] and post-treatment [205] can reduce cerebral infarct size while Danshen post-treatment can also improve regional cerebral blood flow (rCBF) in the ischemic hemisphere and inhibit platelet aggregation in rats [205].

Zhou et al. have well covered the chemistry and pharmacology of Danshen [196]. Usually extracts in TCM herbs are aqueous extracts. However, for Danshen, the preparation is traditionally made with wine and fat for increasing the content of fat-soluble substances [190]. Recent studies show that Tanshinones are the major lipid soluble pharmacological constituents of Danshen, giving Danshen the reddish color and are selectively enriched in the traditional Danshen preparation [206]. With administration of tanshinones IIA (TsIIA) and IIB (TsIIB), there is a reduction in brain infarct volume accompanied by a significant decrease in the observed neurological deficit [190].

The evidence points to the possible role of Danshen in the prevention and treatment of cerebral ischemia [196, 207]. It appears that multiple pathways such as anti-atherosclerosis, anti-hypertension, anti-platelet aggregation as well as anti-inflammatory and anti-oxidative activities are involved [208, 209]. Some clinical trials of Danshen treatment in ischemic stroke have been carried out [196, 207, 210, 211]; yet, the quality of these trials is poor with minimal/no reliable conclusions [210, 212]. Large randomized controlled trials and further scientific research of high quality must be performed to ensure the safety and efficacy of Danshen in treatment of ischemic stroke [196, 212].

Schisandra Chinensis

In TCM, the fruit of *Schisandra chinensis* is used as an anti-tussive, tonic and sedative agent to improve liver and kidney function [27]. Similar to other herbs, *S. chinensis* can inhibit thiobarbituric acid reactive substances (TBARS) formation *in vivo* [213]. Several active components have been identified; these include schisandrin derivatives as well as lignans-schisandrol A, B and C [27]. Pre-treatment of schisandrin B provides cardioprotection in isolated-perfused rat hearts, may be by modulating the I/R-induced changes in non-enzymatic antioxidant levels [214]. Moreover, lignans extracted from *S. chinensis* attenuates the neurotoxicity induced by L-glutamate in primary cultures of rat cortical neurons,

suggesting that *S. chinensis* may possess therapeutic potential against oxidative neuronal damage induced by excitotoxin [215].

Scutellaria Baicalensis

The root of *Scutellaria baicalensis* Georgi (Huang-Qin) is commonly used in TCM as an anti-inflammatory drug and smooth muscle relaxant. It has also been used to treat stroke based on its ability to clear away internal heat [216]. In a rat transient global ischemia model, the methanol extracts from the dried roots of *S. baicalensis* Georgi significantly protect CA1 neurons and inhibit microglial TNF α and NO production. These extracts also protect PC12 cells from H₂O₂-induced toxicity *in vitro* [216].

Previous investigations confirmed that the active components in the root of *S. baicalensis* Georgi are flavonoids [217]. There are 4 major flavonoids, namely wogonoside and its aglycone wogonin as well as baicalin and its aglycone baicalein. The first flavone isolated from its root was wogonin. Wogonin is present only in small amounts in the root while baicalin predominates by far [216]. Out of these 4 flavonoids, baicalin and baicalein can effectively protect SY5Y cells from H₂O₂-induced injury while the effect of wogonin is marginal and wogonoside shows no effect [217].

In cultured primary neurons exposed to either glutamate stimulation, both baicalin and baicalein significantly reduce glutamate-increased lactate dehydrogenase (LDH) release, in which baicalein is much more potent than baicalin. Glutamate-increased intracellular Ca²⁺ is also significantly attenuated by baicalin and baicalein. In neurons exposed to glucose deprivation, baicalein but not baicalin show significant protective effects [218]. These results suggest that baicalein is the most effective compound. Baicalein also displays a radical scavenging effect *in vitro* and a neuroprotective effect *in vivo* by inhibiting hippocampal neuronal death in gerbils after transient cerebral ischemia [219]. On the other hand, baicalin administration can attenuate the elevations of glutamate and aspartate after MCAO in rat, suggesting that baicalin may act as a neuroprotectant during cerebral ischemia [220].

Wogonin has been reported to be neurotoxic in the brain [218]. However, *in vitro* studies showed that wogonin inhibits LPS-induced activation of cultured microglia, leading to a reduction in microglial cytotoxicity toward co-cultured PC12 cells, thereby supporting a neuroprotective role for wogonin [221]. In both experimental brain injury models of transient global ischemia and excitotoxic injury, wogonin attenuates hippocampal neuronal death and the neuroprotective effect is associated with inhibition of microglial activation [221].

Sophora Japonica L.

The dried flowers and buds of *Sophor japonica* is a TCM that can cool blood, stop bleeding and most often used to treat hemorrhoids with bleeding [222]. It is also used in Japan and Korea to treat hemorrhoids and hematemesis [223]. There are numerous components in *S. japonica*; the main ones include flavones, tetraglycosides, isoflavones, isoflavone tetraglycosides, triterpene glycosides, phospholipids, alkaloids, amino acids and polysaccharides [224]. *S. japonica* also contains five main flavonoids of rutin, quercetin, isorhamnetin, isorhamnetin, genistein and kaempferol [224]. Previous studies show that *S. japonica* can reduce the cerebral infarction area and neurological deficit induced by I/R in rats, together with a significant reduction in ED1 and IL-1 β immunoreactive cells as well as apoptotic cells, suggesting that *S. japonica* may reduce cerebral infarction partly as a result of

its anti-inflammatory activities [225]. It is later found that polysaccharides of *S. japonica* scavenge hydroxyl and superoxide anion free radicals [226], suggesting an anti-oxidative effect of *Sophor japonica*. Further studies demonstrate the effects of *S. japonica* on platelet aggregation and cardiovascular function, suggesting its potential as a treatment for cerebral infarct in humans [223, 227].

***Stephania Tetrandra* S. Moore**

The dried root of *Stephania tetrandra* S. Moore, *Radix Stephaniae tetrandrae*, contains an active ingredient, tetrandrine. Tetrandrine has a Ca^{2+} antagonizing property. Pre-treatment with tetrandrine has protective effects against I/R brain damages in gerbils [228]. In rats with MCAO-induced I/R injury, tetrandrine inhibited neutrophilic recruitment, expression of ICAM-1 mRNA and activation of NF- κ B [229].

Herbal Prescriptions in TCM

In TCM, the medicine is usually given to patients in formulas or prescriptions containing a mixture of herbs, with either main or accessory roles. Examples of some well-known prescriptions are described below. They have been shown to display safety and efficacy as well as neuroprotective effects both *in vivo* and *in vitro*.

***Buyang Huanwu Decoction (BYHWD)* 补阳还五汤**

Buyang Huanwu decoction (BYHWD) is a famous classic TCM formula for improving neurological functional recovery from stroke-induced disability. It is created by WANG Qing-ren (王清任) [230] and has been used in China for over 300 years [231]. BYHWD is composed of Astragalus root (*Astragalus membranaceus* in Latin, Huangqi in Chinese, from the family Fabaceae); Chinese Angelica root (*Angelica archangelica* in Latin, Danggui in Chinese, from the family Apiaceae); Red Peony root (*Paeonia lactiflora* in Latin, and Chishao in Chinese, from the family Paeoniaceae); Szechuan Lovage root (*Rhizoma ligustici Chuanxiong* in Latin, Chuanxiong in Chinese, from the family Umbelliferae); Peach Seed (*Semen persicum* in Latin, Taoren in Chinese, from the family Rosaceae); Safflower (*Gnecos* in Latin, Honghua in Chinese, from the family Asteraceae); and Earthworm (*Lumbricus* in Latin, Dilong in Chinese, from the family Lumbricidae). These components are mixed in the above order the ratio of 120:6:6:3:13:3:3 (dry weight) [232].

Numerous studies including clinical trials as well as *in vivo* and *in vitro* experiments have been conducted to further confirm the efficacy of BYHWD treatment and to elucidate the mechanism of BYHWD-mediated neuroprotection. In clinical trials, BYHWD can improve the metabolic imbalance of endothelin and calcitonin gene related peptide in aptients with early cerebral infarction [233]. More importantly, BYHWD can improve neurological function and quality of life in patients convalescent from cerebral infarction [234]. In animals after experimental stroke, BYHWD is beneficial in improving neurological recovery of neurological function [235] and reducing cerebral damages [232, 235-249]. Some mechanisms of BYHWD-mediated neuroprotection have been proposed. For example, heat shock protein 70 (hsp70) upregulation was decreased by BYHWD treatment [238]. BYHWD

could also regulate the expression of glial fibrillary acidic protein (GFAP) in astrocytes [239]. Inhibition of caspase expression [240, 244], neuronal nitric oxide synthase (nNOS) activity [243] and ROS [241] is also observed with BYHWD treatment. BYHWD has also been shown to increase 5-bromodeoxyuridine (BrdU) and therefore promote neurogenesis in the ischemic brain [235, 245]. In addition, BYHWD inhibits the increase in blood-brain barrier permeability and water content, suggesting its modulation on cerebral micro-circulation after ischemia/reperfusion [248]. BYHWD is also suggested to protect the brain from ischemic injury by modulating the expression of pre-inflammatory mediators such as IL-1 β and TNF α [246]. Moreover, BYHWD could reduce I/R brain injury via inhibition of the thioredoxin system [250], [Ca²⁺] [251], free radical scavenger activity [252], and glutamate release [232]. An extensive study using integrative neurofunctional and genomic approaches further showed that BYHWD protects mice against stroke and extend their lifespan through its effects on a variety of genes. These include down-regulation of genes involved in inflammation, apoptosis, anti-angiogenesis and blood coagulation as well as upregulation of genes mediating neurogenesis and nervous development [249]. *In vitro* experiments show that BHD reduced apoptosis [253] by downregulating p53 and p21 gene expression [254] in cultured rat cortical neurons after hypoxia. BYHWD can also protect cultured Schwann cells from H₂O₂-induced oxidative injury, suggesting a possible association of BYHWD-mediated promotion of neural regeneration with its anti-oxidative activity [255]. Taken together, all of these evidences point to multiple mechanisms in BYHWD-mediated neuroprotection in brain ischemia.

Buchang Naoxintong Jiaonang (BNJ) 步长脑心通胶囊

The ingredients of Buchang Naoxintong Jiaonang (BNJ) include Radix astragali, Radix angelicae sinensis, Radix Paeoniae Rubra, Radix Salviae miltiorrhizae, Rhizoma chuanxiong, Semen persicae, Flos carthami, Resina Olibani, Myrrha, caulis spatholobi, Radix achyranthis bidentatae, Ramulus cinnamomi, Ramulus mori, Pheretima, scorpio, and Hirudo. In fact, BNJ consists of BYHWD plus Scorpio and Hirudo; it is therefore a compound preparation of BYHWD [256]. BNJ is an approved TCM for stroke [257]; it is widely used and is well tolerated. BNJ combined with aspirin can enhance the anti-platelet effect in patients with cardio-cerebrovascular diseases [258]. In a randomized, double-blind clinical trial involving 80 patients with apoplexy at convalescence stage, BNJ treatment improves the patients' daily abilities, blood fat and hemorheological indices, suggesting that BNJ has good efficacy in these patients [259]. BNJ also improves the viability of hypoxic primary cortical neurons and significantly reduces neuron apoptosis, suggesting that BNJ can protect cortical neurons from hypoxia-induced apoptosis [260].

Danqi Piantang Jiaonang (DJ) 丹芪偏袒胶囊

Danqi Piantang Jiaonang (DJ) is a TCM drug currently approved in China to improve stroke recovery and is widely available in hospitals in China. DJ is marketed in China as Danqi Piantan Jiaonang and internationally as NeuroAid. It has been evaluated in clinical trials, but the results were only partly published in Chinese medical literature [261]. In 2006, DJ was selected by the Chinese Ministry of Science and Technology as one of the projects in the Key Technologies Research and Development program that aimed at promoting the development of the most promising Chinese innovations [262]. A recent pooled analysis of 2

randomized double-blinded, controlled trials comparing the efficacy and safety of DJ and Buchang Naoxintong Jiaonang (BNJ, see above) was performed. The results show that DJ displayed good tolerability and superiority over BNJ, another traditional Chinese medicine also approved for stroke [263]. Yet, a large Phase III double-blind randomized, placebo-controlled trial of DJ is essential before such treatments can be recommended for general clinical use.

Huang Lian Jie Du Decoction (HHD) 黄连解毒汤

Huang Lian Jie Du decoction (HHD) is also known as Hwangryunhaedok decoction. There are 4 herbs in HHD; they are *Coptidis rhizoma*, *Scutellariae radix*, *Phellodendri cortex*, and *Gardeniae fructus*. It is usually used as a traditional herbal medicine for sedative activity, and for its hypotensive and anti-bacterial effects [264]. Recently, the involvement of HHD in neuroprotection is reported [265-268]. Oral pre-treatment of HHD once daily for 5 days protects against the impairment of learning and memory induced by transient cerebral ischemia. More importantly, HHD significantly protects against cerebral ischemia-induced reduction in the ACh content of the cerebral cortex, hippocampus and striatum, suggesting that the protective effects of HHD may be associated with preventing the decrease in the ACh content of the mouse brain [266]. In addition, oral HHD treatment for five weeks protects against ischemic neuronal death after transient forebrain ischemia by increasing the expression of Cu/Zn-SOD, suggesting that HHD may reduce the exposure of hippocampal neurons to oxidative stress [265]. In a rat model of cerebral I/R, the total infarction volume in the ipsilateral hemisphere is significantly lowered by treatment with HHD [267]. HHD also significantly inhibits MPO activity, an index of neutrophil infiltration in ischemic brain tissue [267]. Out of the four components in HHD, *Scutellariae radix* displays much higher inhibition on total infarction volume and MPO activity than that of the others. It is therefore suggested that *Scutellariae radix* plays a crucial protective role in ischemia-induced brain injury [267]. The results also suggest that the effect of *Scutellariae radix* is the result, in part, of baicalein, a compound contained in *Scutellariae radix* [267].

Kueichih Fuling Wan (KFW) 桂枝茯苓丸

Kueichih Fuling Wan (KFW) is a TCM prescription containing 5 herbal components: *Cinnamomi ramulus*, *Poria cocos* (Schw.) Wolf, *Prunus persics* (L.) Batsch, *Paeonia suffruticosa* Andr., and *Paeonia lactiflora* Pall. It is also known as Keishibukuryogan or Gui-zhi-fu-ling-wan. KFW is originally introduced by Chang Chung-Ching (張仲景) in his Chin-Kuei-Yao-Lueh (金匱要婦人妊娠證並治篇). It is found to be effective in the treatment of cerebral vascular disease in clinical applications [269]. It has been used to treat cerebral ischemia [191].

In rat cerebral ischemia/reperfusion, KFW helps to alleviate the injury [270] as well as increase SOD activity, reduce MDA content and relieve neurological deficits [271]. KFW can also inhibit microglial activation and IL-1 β release and reduce cerebral infarction size in rats with MCAO. [272]. In ischemic neuronal SHSY-5Y cells, KFW can also prevent ROS increase and mitochondrial membrane potential (MMP) decrease. KFW can reduce lipid

peroxide formation and limit oxidative low density lipoprotein (LDL) modification, thereby preventing the progress of atherosclerosis in cholesterol-fed rabbits [273].

Qingkailing (QKL) Injection 清开灵注射液

In the 1970s, the Beijing University of Chinese Medicine modified a TCM, An Gong Niu Huang Wan (安宫牛黄丸), which is composed of *Radix isatidis*, *Flos Lonicerae*, *Concha Margaritifera Usta*, baicalin, *Fructus gardeniae*, cholic acid, hyodeoxycholic acid, and *Cornu Bubali*, and prepared Qingkailing (QKL) injection (清开灵注射液) [274]. In December 1992, QKL was listed in the ‘Practice Guide- line of Essential TCM Patent Drugs in Emergency Department of TCM hospitals, version 1’ [275].

Since then, the use of QKL has grown steadily. It has been extensively used to treat the acute stages of cerebrovascular disease and has shown beneficial effects in improving neurological function [276]. QKL injection has shown diverse pharmacological effects in animal studies, including reduction in Ca^{2+} overload, regulation of matrix metalloproteinase-9 expression and inhibition of inflammation [277-279]. Several clinical studies such as case reports and randomized clinical trials have reported the effectiveness of QKL injections, but no definite conclusion on efficacy and adverse events associated with QKL injection can be drawn after pooled analysis [280]. The lack of adequate evidence in safety assessment also limits the clinical use of QKL [281].

Later a refined formula of QKL, Refined Qing Kai Ling (RQKL), was developed. RQKL is an improved formula derived from the combination of effective and safety components in Qing Kai Ling injection [282]. It consists of cholic acid, hyodeoxycholic acid, baicalin, and jasmninoidin [283]. RQKL is neuroprotective by relieving the damage of vascular endothelial cell and inhibiting the inflammation [284] as well as promoting endothelial nitric oxide synthase expression [282].

Further investigations showed that RQKL can prevent ischemic-induced brain injury with a time window of 6 h, may be by suppressing ER stress-mediated apoptotic signaling [283].

Qizhu Tang Decoction (QZT) 芪术汤

Qizhu Tang (QZT) consists of four herbal components: *Rhizoma atracylodis macrocephalae*, *Poria cocos*, *Radix notoginseng*, and *Radix astragali seu hedysari*. It has been demonstrated to be effective in preventing cerebral oxidative injury in rats [285, 286]. It demonstrates marked anti-oxidative activities *in vitro* by scavenging DPPH and hydroxyl radical as well as inhibiting TBARS formation [285]. When the QZT decoction is injected into rat duodenum 2 hour before cerebral ischemia, TBARS formation and loss of GPx activities are inhibited, suggesting a prevention of oxidative brain damage [286].

Shengmai San 生脉散

Shengmai San (SMS) contains 3 herbal components: *Panax ginseng*, *Ophiopogon japonica* s and *Schisandra chinensis*. It is used mainly for treating coronary heart disease [287]. SMS is first shown to be effective in protecting the rat from cerebral ischemia/reperfusion injury by inhibiting brain edema, intracellular enzymes release, lactate accumulation and lipid-preoxidation [237]. Later studies showed that SMS pre-treatment

inhibits TBARS formation and loss of GPx activity in a rat model of forebrain I/R, thereby preventing cerebral oxidative damage [213, 288]. The anti-oxidant property of SMS is further shown in PC12 cells [289]. SMS pre-treatment also protects rats against heat stroke-induced arterial hypotension and cerebral ischemia by inhibition of iNOS-dependent production of NO and prevention of excessive accumulation of inflammatory cytokines in the peripheral blood stream [290]. These results indicate the potential of SMS as a stroke therapy by inhibiting oxidative damage and preventing cell death.

Acupuncture

In China, more than 30% of patients experienced stroke attack have been treated with traditional medicine, either with traditional Chinese herbs or acupuncture [291]. Many *in vivo* studies have demonstrated that acupuncture could induce either local or long-distance biochemical effects by stimulating sensory neurons [292]. In the basic studies, Zhang et al. reported an interesting observation on Sprague–Dawley rats that were induced to have cerebral ischemia. He treated the rats with electro-acupuncture at the acupuncture point *Baihui*, somewhere located at the cross point of the sagittal mid-line and the line connecting the ears, and used magnetic resonance imaging to examine cerebral edema. It was found that electro-acupuncture could alleviate cerebral edema following the ischemic injury [293].

In clinical studies, Cheng et al. chose 60 patients with post-stroke hand dysfunction and compared the therapeutic effects of acupuncture with routine medicine and physical therapy. Improved scores of hand function and walking ability are found in the acupuncture group, indicating the enhancement of living quality by acupuncture [294]. Hsing's group observed 62 patients with ischemic stroke and treated them with subcutaneous acupuncture over the scalp. They found an improved functional recovery with the acupuncture treatment when compared with the sham group [295]. Several systematic reviews on the safety of acupuncture for ischemic stroke have been published [296–298]. Park et al. concluded that there is no compelling evidence to show that acupuncture is effective in stroke rehabilitation [296]. A later report by Sze et al. showed that with stroke rehabilitation, acupuncture has no additional effect on motor recovery but has a small positive effect on disability [297]. The analysis by Zhang et al. concluded that acupuncture appears to be safe but without clear evidence of benefit [298]. All 3 analyses agreed that larger clinical trials with better study design are essential.

TRADITIONAL CHINESE MEDICINE – WEAKNESS

In Western medicine, industrially manufactured pharmacological drugs are used. In TCM, unlike the Western medicine, extracts and prescriptions are mostly used in which the active ingredients and components are usually not identified, specified and measured. This has led to great variations among batches of herbal medicine, resulting in the difficulties in evaluating and comparing safety and efficacy in clinical trials [299]. Moreover, the results of most TCM clinical trials are published in local non-English journals, making their retrieval and appraisal more difficult. The problem is further worsened by the poor quality of most of

these reports [300]. In 2007, Wu et al. presented the first meta-analysis on the safety and efficacy of 59 traditional Chinese patent medicine (TCPM) listed in the Chinese National Essential Drug (CNED) of 2004 (<http://www.sda.gov.cn>) and those commonly used in China for ischemic stroke [301]. It was concluded that the methodological quality of most included clinical trials was “poor” and there was insufficient evidence of good quality on the effects of TCPM in the treatment ischemic stroke.

TRADITIONAL CHINESE MEDICINE – FUTURE DEVELOPMENTS

In light of the growing interest in TCM for the treatment of ischemic stroke, there is an urgent need in large-scale, properly designed randomized controlled trials followed by appropriate meta-analysis [299]. Since the use of TCM in a complex intervention in diseases, the study options suggested by Campbell and colleagues can be used [302]. The recently developed CONSORT guidelines are also suggested [299, 303, 304].

In addition, the Government of the People’s Republic of China has played major roles in defining the scientific principle and position of TCM. Numerous measures are being undertaken [305]. Recently, the Herbalome Project was formally proposed at the 103rd Eastern Forum of Science and Technology in 2007, providing a new theoretical basis for TCM research. Through the Herbalome Project, one can elucidate the constitution, structure and function of the herbs in TCM. A resource library of the various herbal substances will also be built. Most importantly, it should be noted that the research of TCM should not be considered as identical to that in Western medicine [305].

CONCLUSION

Stroke is a devastating disease and causes major social burden worldwide. Despite the vast diversity of investigations using *in vitro* and *in vivo* experimental models as well as clinical trials, the lack of effective and widely applicable pharmacological treatments for ischemic stroke patients persists. Many people have turned to the traditional medicine that has abundant clinical data as well as extensive observational and anecdotal experiences in the hope of identifying effective treatments. Experience on the usage of TCM has been inherited and accumulated over thousands of years and has been used in treatment of ischemic stroke. Its safety and efficacy, although not proven according to the present standards, should not be doubted. Yet, further scientific research of high quality as well as large-scale, properly designed randomized controlled trials followed by appropriate meta-analysis are necessary and essential before such treatments can be recommended for general clinical use.

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Chapter 10

ISCHEMIC STROKE IN CHILDREN: SYMPTOMS, PREVENTION AND RECOVERY

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ABSTRACT

Arterial ischemic stroke (AIS) is a rare neurological disorder among the pediatric population. Its long-term sequelae include motor and sensory impairments, behavioural and psychiatric problems, cognitive deficits and epilepsy. Clinicians treating pediatric AIS are often presented with significant management challenges due to mistaken or delayed diagnosis and the need for better evidence regarding its management. Long-term sequelae of pediatric AIS include motor and sensory impairments, cognitive deficits, behavioural and psychiatric problems and epilepsy.

Current therapeutic strategies for pediatric AIS focus on supportive therapy and secondary prevention with anticoagulation or anti-platelet therapy in those with increased risk of recurrence. In children with AIS, the role of fibrinolytic therapies, such as tissue plasminogen activator, in the management of acute stroke remains unclear due to the lack of randomized control trials and lack of evidence for its safety and efficacy in pediatric populations. As such, comprehensive studies to guide the management of pediatric AIS are critically needed to improve outcomes as they present significant morbidities to both patients and their families. In this chapter, the current literature with regards to the risk factors and etiologies of pediatric AIS such as congenital heart disease, sickle cell disease, arteriopathies and prothrombotic disorders will be reviewed. In addition, maternal and fetal risk factors such as infertility, placental abnormalities and infection will be discussed as they have been found to be associated with neonatal AIS. Finally, the various clinical presentations and outcomes of pediatric stroke will be summarized, focusing at large on the importance of recognizing signs and symptoms of AIS well in advance for clinicians to administer prompt diagnostic and treatment interventions and not be faced with challenges of missed or delayed diagnosis.

INTRODUCTION

Ischemic stroke is one of the many principal causes of death and disability in children. In the past, ischemic stroke was considered to be a rare occurrence in children when compared to the adult population. However with time and improved understanding and advances in neuroimaging techniques, proper recognition and diagnosis of stroke in children has been accomplished. Multiple causes and risk factors are implicated in the etiology of ischemic stroke in children.

Ischemic stroke that occurs during childhood can be categorized as either childhood stroke (occurring between 1-month and 18-years of age) or ischemic perinatal stroke (occurring between 20-weeks of fetal life through the 28th postnatal day). Notably, the timeline of occurrence for ischemic perinatal stroke can be divided into three categories. The first category being fetal ischemic stroke, diagnosed before birth by using fetal imaging methods or in stillbirths based on neuropathologic examination. The second is neonatal ischemic stroke diagnosed after birth and on or before the 28th postnatal day including preterm infants. Finally, the third being presumed perinatal ischemic stroke, diagnosed in infants more than 28 days of age in whom it is presumed but not certain that the ischemic event occurred sometime between the 20th week of fetal life through the 28th postnatal day [1] (Figure 1).

As in adults, the ischemic stroke subtypes in children include: arterial ischemic stroke (AIS) that is caused by occlusion or stenosis of the cerebral arteries and cerebral sinovenous thrombosis (CSVT) that is caused by occlusion of the cerebral veins or venous sinuses. In adults, the development of standardized AIS classifications and terminologies has greatly helped clinicians and researchers to determine outcomes, recurrence risk and treatment choices for different AIS subtype. For childhood AIS, no such classification system existed until recently, when a consensus based classification system for childhood AIS was developed for the first time. This classification system, known as the Childhood AIS Standardized Classification And Diagnostic Evaluation (CASCADE) criteria, was developed by a group of international pediatric stroke study investigators to standardize and facilitate both clinical care and data collection methods for childhood AIS [2]. In addition, to facilitate research in cerebrovascular disorders, the National Institute of Neurological Disorders and Stroke recently supported an initiative that led to the development and publication of the stroke-specific Common Data Elements (CDEs) for both adults and children [3].

In the present chapter, ischemic stroke subtypes in the pediatric age group are reviewed with particular emphasis on the clinical presentation, causative and risk factors, treatment and preventative strategies and outcomes in children with ischemic stroke.

ARTERIAL ISCHEMIC STROKE

Arterial ischemic stroke (AIS) is defined by the presence of persistent focal neurological deficit of acute onset and neuroimaging evidence of focal parenchymal infarct or infarcts conforming to corresponding arterial territory (Figure 2). These focal neurological deficits in infants can be seizures alone or other signs of encephalopathy e.g., extreme irritability or alteration in consciousness. In some children, focal neurological deficits are transient, lasting

less than 24-hours. When there is no neuroimaging evidence of new focal area(s) of infarction and the event is not due to other etiologies (e.g., seizure, migraine) the event is then considered to be a transient ischemic attack (TIA).

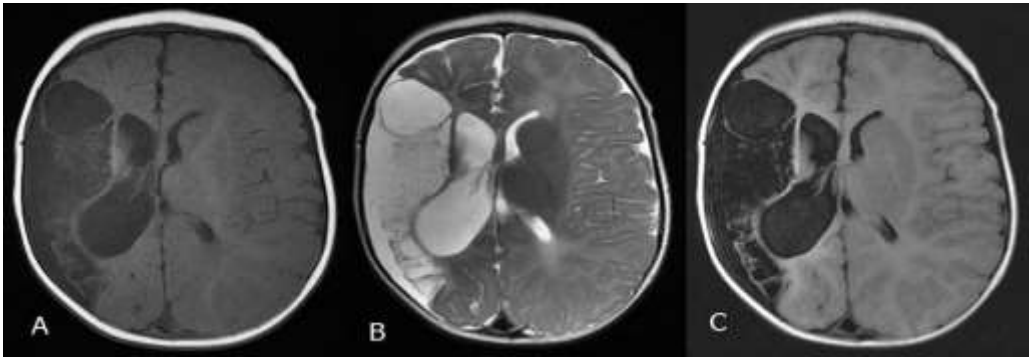


Figure 1. A 6 months old infant with presumed ischemic perinatal stroke who presented at the age of 6 months with infantile spasms and evidence of right sided pathologic handedness and left moderate hemiparesis. A, B and C) MRI brain on axial T1, T2 and FLAIR sequences demonstrating cystic gliosis and ex-vacuo dilatation of the right lateral ventricle in the territory supplied by the right middle cerebral artery.

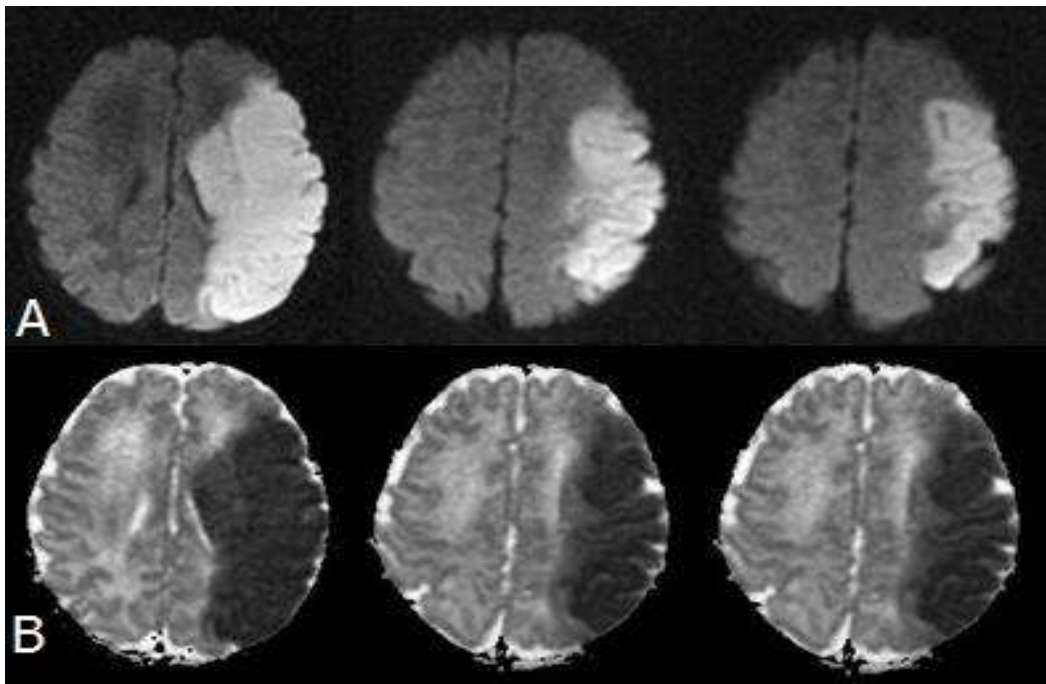


Figure 2. A term infant with complete acute left middle cerebral artery territory ischemic stroke who presented few hours after birth with apneas and right hemibody focal seizures. A and B) Axial diffusion weighted MRI brain demonstrating diffusion restriction in the left frontal and parietal lobe regions and basal ganglia with corresponding apparent diffusion coefficient map consistent with an acute ischemic infarction involving the left middle cerebral artery territory.

In children, the reported incidence of AIS ranges from 0.6 to 7.9 per 100,000 children per year [49] with higher incidence among males and black Africans [10-12] (Table1). Over half of the strokes occur in children less than one year of age [8]. The estimated incidence varies widely depending upon the types of studies that are conducted. Population based studies have reported lower incidences whereas studies based on hospital discharge databases have shown higher incidences [6, 13-16]. Schoenberg and colleagues were the first to report a population based incidence of 0.6 per 100,000 children per year [16]. Subsequent population based studies have reported increased incidence rates for AIS in children ranging from 2.6 to 7.9 per 100,000 children per year [6, 13-15]. In the United States, a national study of hospital discharge database for children with ischemic stroke, revealed an estimated incidence of 7.8 per 100,000 children per year [8]. In Asia however, studies based on hospital database have estimated much higher incidences, up to 29.7 per 100,000 children per year [4]. Irrespective of the type of study, over time, pediatric literature has indicated an overall increase in the incidence of childhood AIS. This apparent increase in incidence is likely related to increased awareness of the disorder among clinicians, improvements in imaging techniques used for the diagnosis of stroke, increasing survival in previously fatal disorders and the increased use of therapeutic interventions to treat most of these disorders (such as complex congenital cardiac conditions). Additionally, the increased incidence seen in reports based on hospital discharge databases is likely due to the fact that most of these hospitals serve as tertiary care centers and have large catchment areas that serve multiple regions within the state or the country.

CLINICAL PRESENTATION

The clinical presentation of AIS in children is related predominantly to the age of the child and the location of the infarction. The onset of symptoms is variable, either with abrupt onset or with stuttering symptomatology. However, in majority of children the symptoms are subtle and often non-specific. Even if children present with stroke specific symptoms such as focal neurological deficits, the presenting symptoms are often attributed to other neurological disorders that are commonly seen in children, such as seizures with ictal or post-ictal Todd's paresis, complicated migraine, intracranial tumor or hemorrhage, infection or demyelination. In addition, seizures, fever, lethargy and headache are more common in children with AIS [17, 18]. As a result, the diagnosis of ischemic stroke in children is often missed and not considered as the first diagnostic consideration by the frontline medical and paramedical staff.

Young infants with acute ischemic stroke usually present with seizures, irritability or altered consciousness. Presentation with hemiparesis may be present but is often difficult to recognize in this age group. Toddlers typically present with acute neurological deficit (usually hemiparesis and/or facial droop) and seizures. Older children can present with other neurological deficits such as speech, visual, focal sensory abnormalities, ataxia or headache in addition to hemiparesis and facial weakness [14, 19].

As in adults, the presentation of ischemic stroke in children differs based on the location of ischemic infarction which is determined by the vascular territory and the circulation involved. The areas of the brain supplied by the internal carotid arteries and their branches (main branches include middle and anterior cerebral arteries) comprise the anterior circulation and the areas supplied by the vertebrobasilar system (vertebral and basilar arteries and their

branches) constitute the posterior circulation. Children with infarction in the middle cerebral artery territory typically present with sudden onset hemiparesis with or without facial weakness or speech abnormalities. Anterior cerebral artery territory events usually present with predominant contralateral leg weakness compared to arm or leg weakness alone. In addition to the contralateral hemiparesis, infarcts isolated to the basal ganglia can present with contralateral movement disorders, such as hemidystonia or chorea [20]. Posterior circulation ischemic strokes are less common in children and account for less than 10% of ischemic stroke cases [21]. Visual deficits are common with posterior cerebral artery territory events or with involvement of the optic pathway. Vertebral and basilar artery ischemic strokes typically present with vomiting, vertigo, headache, alteration in consciousness, coordination difficulties, hemiparesis or asymmetric quadriparesis and papillary, oculomotor and bulbar manifestations [21, 22].

Table 1. Annual Incidence Stroke in Children

Studies	Year Reported	Annual Incidence of Stroke [†]	Arterial Ischemic Stroke Incidence	Number of Stroke Patients
USA (Rochester) [16]	1978	2.5	0.6	4
USA (Cincinnati) [13]	1993	2.7	1.2	16
Europe (Dijon) [15]	1995	13	7.9	28
Iran (Khorasan) [7]*	1999	1.8	17	17
USA [8]*	2002	11.9	7.8	1377
USA (California) [11]*	2003	2.3	1.2	2278
Hong Kong [5]*	2004	2.1	36	50
Canada [6]	2006	0.7	2.6	820
Saudi Arabia (Al-Khobar) [4]*	2007	29.7	28	31

* Based on hospital admission and discharge database.

[†] Cases per 100,000 children population per year for both hemorrhagic and ischemic strokes.

Although the majority of children with AIS present with a single episode of focal neurological deficit, recurrent transient or minor ischemic events may be present in about one third of children that later present with a major ischemic event or fixed neurologic deficit. In children, prompt evaluation with neuroimaging is important since TIA recognition and differentiation from other disorders with similar brief neurological events such as seizures and migraines can be clinically challenging [23].

For children presenting with symptoms suggestive of AIS diagnosis, early and specific parenchymal neuroimaging is required to differentiate from other neurologic conditions and establish a definitive AIS diagnosis. The head computed tomography (CT) has easy accessibility as well as ability to readily rule out intracranial hemorrhage and mass lesions. It is for these reasons that head CT scan is often the first neuroimaging test that is completed in children that are suspected of AIS or other focal brain lesions. However, compared to magnetic resonance imaging (MRI), CT scan has low resolution and may miss the signs of early infarction, especially when done within 12 hours of ischemic stroke symptom onset. When available and feasible, brain MRI with diffusion weighted sequences is the specific and

preferred neuroimaging test for the diagnosis of AIS in children. Since identification of an arteriopathic condition or occlusive clot will determine initial treatment intervention, it is advised that in all clinically suspected AIS cases, non-invasive vascular imaging, utilizing either CT or MR angiography, should be included in the initial neuroimaging work-up. Subsequent to the initial diagnostic neuroimaging test, selected cases may require invasive conventional catheter cerebral angiography, particularly when initial non-invasive vascular imaging suggests vascular occlusion, arteriopathy or is inconclusive of these diagnoses.

Etiologies and Risk Factors

Once the AIS diagnosis is confirmed, an aggressive and timely search for the underlying etiologies and risk factors for AIS is recommended since conditions that predispose to stroke may themselves require urgent treatment and are closely linked to the risk of AIS recurrence and poor outcomes in children [24, 25]. The etiologies and risk factors of childhood AIS differ significantly from those in adults [19]. In adults, the leading causes of AIS are: atherosclerosis, hypertension, cardiac dysfunction (in particular atrial fibrillation) and small vessel diseases. In children, congenital cardiac disease or cardiac intervention, arteriopathies, central nervous system infections and prothrombotic and hematological disorders, are common identifiable risk factors for AIS. A number of these contributing factors may coexist in a child with AIS [25]. It should also be noted that, in spite of a thorough and systematic search for an underlying etiology, in at least half of the children with AIS, an etiology or risk factor for AIS is not identified [26]. Depending upon the populations studied and breadth of etiological investigations carried out by the different care centers, the reported occurrence of the potential etiologies and risk factors for AIS vary considerably in children. Recent studies have identified risk factors in over 70% of children with AIS [27]. The commonly associated etiologies and risk factors for AIS in children are briefly discussed below and listed in Table 2.

Cardiac Disease or Intervention

Cardiac disease or intervention leading to cardioembolic stroke is one of the most common etiologies for AIS in children. In a prospective population based study and several case series, a cardiac disorder was present in 20 to 28% of children with AIS [6, 25, 26]. In adults, atrial fibrillation, myocardial ischemic dysfunction and valvular disease constitute the common causes for cardioembolic stroke. In children congenital cardiac malformations with prominent intracardiac shunting, cardiomyopathies, arrhythmias and endocarditis are commonly responsible etiologies. The majority of AIS associated with these pediatric cardiac causes occur in the setting of a cardiac surgical procedure or diagnostic and interventional catheterization [26, 28]. In such circumstances, studies have reported highest risk of stroke occurrence in the first few years of life after the cardiac intervention has occurred [29, 30]. Most children with cardioembolic stroke present with sudden or abrupt onset focal neurologic deficits, headache, seizures or alteration in level of consciousness. In over half of the children, the symptoms are identified on awakening, usually from a medical sleep required for a cardiac intervention [31-33].

Table 2. Etiologies and Risk factors for Arterial Ischemic Stroke in Children

Cardioembolic Risk Factors	Prothrombotic Disorders and States
Acquired causes (e.g., dysrhythmia, atrial myxoma, bacterial endocarditis, rheumatic heart disease)	<i>Inherited Causes</i> Protein C deficiency
Congenital cardiac defects (e.g., ventricular hypoplasia, transposition of great arteries, Tetralogy of Fallot, septal defects, valvular defects)	Protein S deficiency Activated Protein C resistance Plasminogen disorders
Cardiac surgery or catheter intervention	Fibrinogen disorders
Hematological Disorders	Factor VII, VIII and XII elevations
Hemoglobinopathy (e.g., sickle cell disease)	Lipoprotein (a) elevation
Iron deficiency anemia	Lupus anticoagulant presence
Polycythemia	Antiphospholipid or anticardiolipin antibody presence
Essential thrombocythemia	Hyperhomocystenemia
Heparin induced thrombocytopenia	Factor V Leiden mutation
Thrombotic thrombocytopenic purpura	Prothrombin 20210A mutation
Vasculopathies	<i>Acquired Causes</i>
Moyamoya (disease, syndrome)	Cancer
Transient cerebral arteriopathy (e.g., post varicella angiopathy, other post infectious arteriopathies, idiopathic)	Procoagulant drugs (e.g., L-asparaginase) Nephrotic syndrome
Craniocervical arterial dissection	Metabolic and Genetic Factors
Traumatic	Organic acidemias
Infectious arteriopathies (e.g., secondary to meningitis, sepsis)	Mitochondrial disorders
Idiopathic focal segmental arteriopathy	Dyslipoproteinemia (e.g., hypercholesterolemia, hyperlipoproteinemia, Progeria)
Fibromuscular dysplasia	Fabry Disease
Radiation induced arteriopathy	Neurocutaneous disorders (e.g., NF1, Sturge Weber)
Diffuse CNS Vasculitis (primary, secondary)	Phaces syndrome
	CADASIL
	Connective tissue disorders(e.g., Ehler Danlos, Marfan)

Cerebral Arteriopathies

Childhood cerebral arteriopathies are disorders of the cerebral arterial wall abnormalities that can be either monophasic or have a progressive monophasic or undulant prolonged course [34]. Childhood cerebral arteriopathies reportedly predispose to AIS in approximately 30% to 60% of older children with AIS [6, 24, 26, 34] with much recent studies reporting higher rates [27]. However, considering that not all children with AIS receive appropriate vascular imaging and some may have false negative non-invasive vascular imaging, the occurrence of arteriopathy in children with AIS is likely underestimated. Arteriopathies in children can be inflammatory (related to infectious, post infectious or autoimmune mechanisms), genetic, structural, toxic or traumatic causes. International collaborations and

efforts are striving to better categorize and define these arteriopathies [2, 34, 35]. Improved and early use of neurovascular imaging in pediatric stroke (including both invasive and non-invasive imaging) over the years has greatly improved our understanding of different arteriopathic conditions. Additionally, recent non-invasive imaging techniques utilizing vessel wall imaging are showing promising findings in better defining and characterizing these arteriopathies [36]. Common arteriopathies associated with AIS in children include moyamoya, arterial dissection, focal cerebral arteriopathy (including transient cerebral arteriopathy of childhood and infectious and post infectious arteriopathies) and diffuse central nervous system (CNS vasculitis), are described below.

Moyamoya disease reportedly affects all ethnic populations, but predominantly Japanese and other Asian populations. It is a rare progressive vaso-occlusive cerebrovascular disorder of unknown etiology. It is characterized by stenosis and occlusion of the arteries at the base of the brain with abnormal tangiectatic collateral circulation [37] (Figure 3). Most often, moyamoya disease is sporadic but some familial clustering is reported. Additionally, moyamoya is seen in association with other medical conditions and is then called as moyamoya syndrome. The disorders commonly associated with moyamoya syndrome include genetic syndromes, (Down's syndrome Allagile syndrome, Williams syndrome), neurocutaneous disorders, post radiation exposure, hemoglobinopathies and renovascular abnormalities [38]. The younger children with moyamoya typically present with recurrent ischemic strokes that are often transient and occur in association with hyperventilation. On the other hand, older children with moyamoya present with hemorrhagic strokes. The clinical symptoms are variable and dependent upon the type and the location of stroke. In moyamoya, focal neurological deficits are common in children with ischemic stroke presentation and signs of raised intracranial pressure are common in children with hemorrhagic stroke presentation, such as alteration in level of consciousness, headache and vomiting.

Craniocervical arterial dissection is a well established etiology for stroke during childhood. The reported frequency varies from 7%- 20% [39-42] with some studies reporting a male predominance [40]. Prominent presenting features include, head and neck pain, alteration in level of consciousness and focal motor neurological deficits. Horner's syndrome, commonly reported in adults, is not frequently noted in children [41]. Head and neck trauma leading to arterial wall tear can occur spontaneously and is the responsible mechanism in over half of the children with craniocervical arterial dissection [39-41]. Apart from trauma, other predisposing factors for craniocervical arterial dissection include connective tissue abnormalities, fibromuscular dysplasia, anatomical variations, infections and hyperhomocysteinemia [40]. Characteristic vascular findings that support a diagnosis of dissection include the following: focal segmental irregular stenosis of a large vessel in the presence of aneurysmal dilatation (pseudo aneurysm), presence of an intimal flap, double lumen and or bright crescent sign (on axial fat saturated T1 sequence of magnetic resonance imaging) or occlusion of a cervical artery or a vertebral artery at either C1-C2 or C2-C3, with a history of preceding head or neck trauma (within two weeks of presentation) [40, 41].

A strong relationship between arteriopathies secondary to infection is noted in children with AIS. Commonly reported infections that may lead to direct arteriopathic abnormalities in children include bacterial meningitis, encephalitis and septicemia, infection with mycoplasma pneumoniae, Coxsackie's B4 and A9, parvovirus B19, influenza A and human immunodeficiency viruses [9, 43, 44]. Some inflammatory focal cerebral arteriopathies occur after an infection and are termed post infectious. Although common in children, the

pathogenic mechanisms for inflammatory focal arteriopathies are poorly understood in children. The examples of these inflammatory focal cerebral arteriopathies include transient cerebral arteriopathy of childhood (TCA) and idiopathic focal segmental arteriopathy. The TCA characterized by a monophasic course and preferential involvement of unilateral distal branches of the internal carotid artery and proximal branches of the anterior cerebral and middle cerebral arteries (the carotid T distribution) (Figure 4). The infectious agents involved with TCA include varicella zoster, Coxsackie's B4 and A9, parvovirus B19, influenza A and enteroviruses. Prior exposure to varicella zoster within the 12 months of onset of TCA (also known as the post varicella angiopathy) is implicated in at least half of the patients with TCA [35, 45].

All other segmental focal arteriopathies with unknown etiopathogenic mechanisms comprise the idiopathic group which is markedly heterogeneous and poorly studied. In addition, it is not uncommon to confuse clinical and radiological presentations of arterial dissection and early onset moyamoya with some the above mentioned focal arteriopathies [35].

Diffuse central nervous system vasculitis is defined as diffuse or multifocal areas of segmental narrowing and dilatation (beading) involving small or medium sized cerebral vessels. The diffuse CNS vasculitis can be either a primary angiitis exclusive to the central nervous system (a well defined angiographic and pathologic entity in pediatric stroke literature or secondary angiitis in association with systemic vascular inflammatory disorders such as systemic lupus erythematosus, polyarteritis nodosa, Behcets disease, Sjogrens syndrome, sarcoidosis, inflammatory bowel disease and a variety of other autoimmune disorders [46-49].

Prothrombotic and Hematological Disorders

Various prothrombotic and hematological disorders have been associated with ischemic stroke occurrence in children. The clinical symptomatology is not unique and is similar to general ischemic stroke presentations. Observation of the family history for these children may reveal presence of a similar disorder or a tendency for one or more of the family members to form clots. Sick cell disease is one of the most common hematological disorders associated with childhood AIS. Over 25% of patients that are homozygous for this hemoglobinopathy develop AIS. Although majority of children with sickle cell anemia suffer from their ischemic stroke events during an acute sickle crises, some children will have their strokes due to a secondary vasculopathy, often moyamoya [28, 50-52]. It is therefore important to monitor all children with sickle cell anemia for both vascular flow studies as well as progressive vascular abnormalities. Other hematological disorders associated with childhood AIS include iron-deficiency anemia [26, 53], polycythemia and thrombocytosis [54, 55].

Hypercoagulable states or thrombophilic disorders, both inherited and acquired, have been reported to occur in 20-68% of children with ischemic stroke [14, 26, 54, 56-58]. Studies have noted that variations in the occurrence of hypercoagulable abnormalities in children with AIS are due to the nature and extent of investigations undertaken, types of stroke investigated, and geographic and ethnic differences between studies [57, 59, 60]. Most commonly noted hypercoagulable abnormalities include: deficiencies of Protein C, Protein S,

antithrombin III and plasminogen; serum elevations of lipoprotein(a) and homocysteine; presence of antiphospholipid antibodies; mutations of factor V Leiden, prothrombin 20210A gene and methylenetetrahydrofolate reductase deficiency (MTHFR). Some of these coagulation abnormalities do not directly cause AIS but rather increase the risk of stroke by combining with other factors that trigger or predispose to AIS [61].

Genetic and Other Risk Factors

Apart from the already described and known genetic disorders that are associated with an increased risk for ischemic stroke (e.g., Fabry disease, Progeria, PHACES syndrome, etc.), an individual's personal and or familial genetic predisposition (either monogenic or polygenic) require further research [62, 63].

Other risk factors for AIS that are uncommon in children and are similar to the adult AIS patients include use of oral contraceptives and/or street drugs, particularly amphetamines and cocaine; personal history of migraines, and metabolic (carbohydrate deficient glycoprotein syndrome, homocystinuria) and lipid disorders [14, 23, 28].

Risk Factors for Ischemic Perinatal Stroke

During the perinatal period, cardioembolic, inflammatory and hypercoagulable conditions are relatively less common risk factors [8]. A number of placental, fetal and maternal factors play an important role in ischemic stroke etiopathogenesis during this period. Commonly reported maternal risk factors associated with perinatal stroke include presence of a prothrombotic condition (mainly positive antiphospholipid antibody status), infertility, primiparity, oligohydramnios, prolonged 2nd stage of labour, preterm labour, delivery via emergency C- section delivery or vacuum extraction, preeclampsia, and prolong rupture of membranes [8, 57, 64, 65]. A variety of placental pathologies are associated with an increased predilection for thrombus formation including vascular necrosis, hypoperfusion, chronic villitis, and, most commonly, chorioamnionitis, (which accounts for at least 10-15% of all live births.) [66, 67]. Fetal risk factors include male child and fetal heart rate and cord abnormalities. Furthermore, presence of multiple risk factors often coexists in the same individual infant, which confers greater risk for stroke in these infants. However, similar to older children, in at least 30% of ischemic perinatal stroke cases an underlying cause or risk factor for AIS occurrence may not be identified [8, 9].

TREATMENT AND PREVENTION OF CHILDHOOD AIS

There are no multicenter or randomized controlled clinical trials for the acute treatment or prevention of childhood AIS, except in children with sickle cell disease. Although systematic approaches to pediatric stroke care and research are emerging, these are still at very early stages. The treatment of childhood AIS is either extrapolated from the adult ischemic stroke literature or based on treatment approaches published in the pediatric case reports and cohort studies. The application and development of therapeutic interventions for stroke in children is therefore faced with many ethical and practical challenges for both the clinicians and the researchers. This led to the emergence of the recently published expert consensus based guidelines for the management of childhood AIS [44, 68-70]. It should be

noted that the evidence for most of the recommendations in these guidelines are class I (evidence or agreement that the procedure or treatment is useful and effective) or class II (conflicting evidence of divergence of opinion about the usefulness/efficacy of procedure or treatment) with level of evidence C (expert opinion or case studies) [44, 69, 70].

The therapeutic and preventative approaches for the treatment of childhood AIS in general as well as specific to some common ischemic stroke etiologies are discussed in the following section.

General Treatment of AIS

As in adults, the treatment of arterial ischemic stroke in children is twofold: acute and long-term preventative treatment.

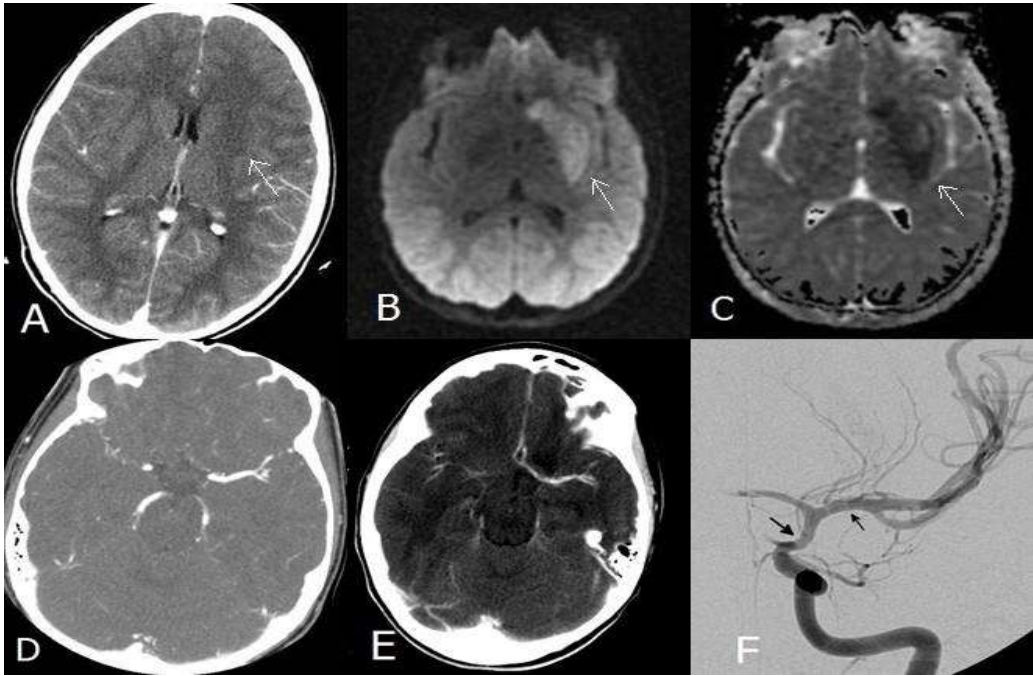


Figure 3. An 11-year-old, previously healthy, right-handed girl with acute left basal ganglia ischemic stroke presented with slurred speech, decreased level of consciousness and right hemiparesis. A) Head computed tomography (CT) 8 hours after initial onset of symptoms revealed hypodensity in the region of the left lentiform nucleus consistent with an acute infarct. B and C) Magnetic resonance imaging (MRI) at 21 hours post-stroke demonstrated diffusion restriction in the left lentiform nucleus with a corresponding apparent diffusion coefficient map (B) in that region. D and E) Her contrast enhanced CT angiogram and MR angiogram were normal. F) A subsequent cerebral catheter angiogram demonstrated narrowing and irregularity in the distal segment of the left internal cerebral artery, proximal M1 segment of left middle cerebral artery and the A1 segment of the left anterior cerebral artery (arrows).

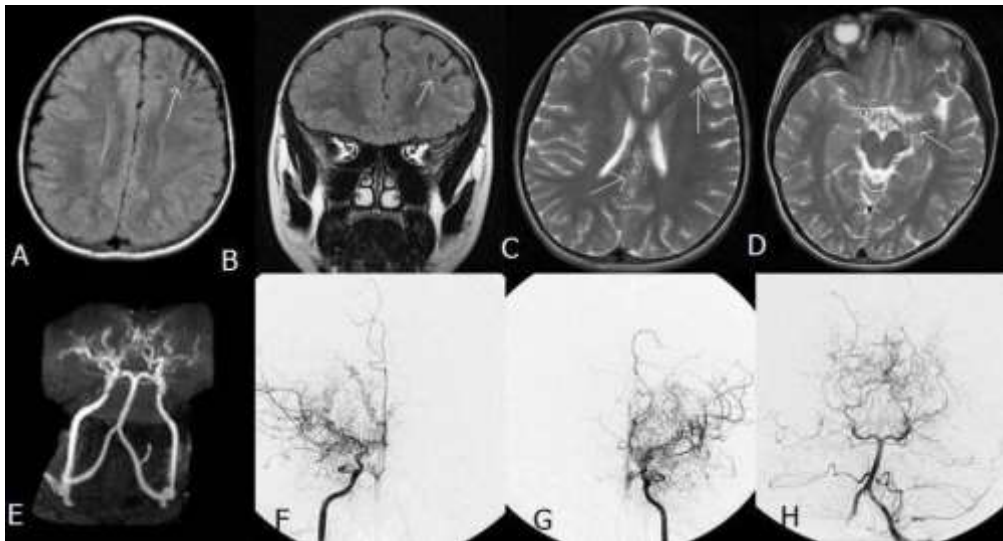


Figure 4. A 10-year-old, right-handed girl with left frontal old ischemic stroke who presented with a long-standing history of headaches with or without associated transient hemisensorimotor symptoms, frequently affecting left hemibody compared to the right side. A and B) MRI brain revealed evidence of volume loss on axial and coronal FLAIR sequences and (C) hyperintensity in the left frontal region on axial T2 sequences (arrows). The axial T2 sequences (C and D) and time of flight MR angiogram (E) reveal evidence of prominent tiny arteries and capillaries in the bilateral basal ganglia and parasagittal regions (arrows). F and G) The catheter cerebral angiogram on right and left internal carotid injections confirmed occlusion of bilateral distal internal cerebral arteries with reconstitution of the middle cerebral artery proximal segments via the deep arterial anastomosis and evidence of capillary collaterals and prominent hypertrophic deep perforating arteries giving the classical picture of moyamoya (or puff of smoke). G) The left vertebral artery injection show similar hypertrophic perforating arteries and new sprouting capillary collaterals.

Acute Treatment

The acute treatment is further categorized into hyperacute and sub-acute treatment. Hyperacute treatment strategies are targeted to the initial 6-hours and the sub-acute treatment to 6-hours to few days following the ischemic stroke symptoms onset.

Hyperacute Thrombolytic Therapy

Over the last two decades, adult literature has exploded with various thrombolytic and neuroprotective therapeutic interventions that may be used for the treatment of AIS during the hyperacute phase (typically within a narrow therapeutic window of 0-6 hours from the symptom onset). [71-73] However, many such therapies in adults are either experimental or not approved as standard of care treatment for AIS. Currently, in adults, systemic fibrinolytic therapy with tissue type plasminogen activator (t-PA or alteplase), given within the 4.5 hours of symptom onset, is the only approved standard hyperacute treatment of AIS. Intravenous administration of t-PA in adults with AIS was first shown to be effective in 2004, in a clinical trial funded by the National Institutes of Neurological Disorders and Stroke. This clinical trial clearly demonstrated that the group of patients who received t-PA within 3- hours of stroke symptom onset had significantly reduced disability from stroke compared to the placebo treated group (with 30% patients more likely to have minimal or no disability 90 days after

the treatment with t-PA), [73]. The trial noted that the risk of symptomatic intracranial hemorrhage was 6.4% when t-PA is instituted within the 3-hours therapeutic window compared with 0.6% in the placebo or the non-tPA treated group [73, 74]. However, a recent study in adults with ischemic stroke has demonstrated both safety and efficacy with the therapeutic window for intravenous t-PA extended to 4.5 hours leading to the recent approval of its use within 4.5 hours of stroke symptom onset in adults [75]. The other commonly practiced and favored hyperacute therapeutic interventions for selected adult stroke patients (although not yet standard of care treatment) is targeted intra-arterial administration of t-PA within 3-6-hours of ischemic stroke symptom onset [76]. This targeted and direct administration of drug into the thrombus reportedly has vascular recanalization rates up to 58% - 66% and an improved clinical outcome at 90 days in 31% - 40% of patients [76]. Other hyperacute interventions that have been studied in adults include clot busting interventions, such as use of mechanical clot retrieval devices (with recanalization rates as high as 57%), and neuroprotective strategies, such as whole body hypothermia or selective head cooling. These interventions have shown promising results but require further studies before these are widely adapted or approved for clinical use in adults with ischemic stroke [71, 72, 77-79].

Current pediatric literature reports either only single cases and few cohort studies or literature reviews/pooled analysis for the use of t-PA in the hyperacute phase of AIS [80-84]. In these studies, treatment with t-PA has reported efficacy rates as high as 50 – 80% in children with AIS, but with bleeding rates as high as 25% -50%. The bleeds were, however, mild and did not require transfusion therapy or any other intervention [85]. Since in children many age-related maturational, pathophysiological and developmental differences exist compared to adults, one cannot entirely extrapolate from adult studies. The development of systematic and randomized controlled studies involving these potentially beneficial hyperacute therapies are, therefore, of utmost importance in children with ischemic stroke. As a result, the much needed clinical trial to test the efficacy and safety of t-PA in children, the Thrombolysis in Pediatric Stroke (TIPS) study, was recently designed and developed, under the umbrella of the international pediatric stroke network. The TIPS study is a multicentre clinical trial approved and funded by the National Institute of Health. This study will systematically evaluate the safety and dose dependent efficacy of intravenous t-PA in children with AIS, aged 2-17 years. The t-PA will be administered intravenously in a peripheral vein within 4.5 hours of the onset of stroke symptoms. The intra-venous t-PA doses tested will have three dose tiers dependent upon toxicity (0.75, 0.9, 1.0 mg/kg), with 10% of the dose to be given as a bolus over 5 minutes and the remaining 90% given over one hour. Inclusion criteria will include clinical presentation with acute onset of neurological deficit in a pattern consistent with arterial territory ischemia with radiological confirmation via head CT or MRI and presence of partial or complete occlusion in an intracranial artery corresponding to the infarct location on CT or MR angiography [86] (and personal communication at the IPSS network meetings).

Although, recent advances in the treatment of pediatric ischemic stroke has resulted in promising avenues, one needs to be mindful of evidence based treatment approaches. All current published guidelines for the management of children with stroke are in agreement in not recommending hyperacute fibrinolytic therapy with t-PA for acute childhood AIS, except in the setting of a clinical trial and until much is known about the safe administration of this medication in children [44, 68-70].

It should also be noted that to be effective and safe, t-PA and other hyperacute treatments must be instituted within a narrow therapeutic window [71-74] requiring prompt and correct stroke diagnosis. Therefore, in adults, hyperacute diagnosis has become the major focus for education campaigns and research. In adults, major advances in acute stroke care have significantly reduced both the pre-hospital and in-hospital delays, delay in arrival to a hospital and receiving timely diagnosis and treatment for acute stroke continues to be a concern in adults [87-93]. In children suspected of AIS, timely and accurate diagnosis of AIS is challenging mainly due to lack of awareness and existence of 'stroke mimics' resulting in delay in AIS diagnosis. Contrary to adults who have significant pre hospital delays, children have marked in-hospital delays in AIS diagnosis (mainly in obtaining assessments and confirmatory diagnostic imaging). In children with AIS, studies report overall delay in AIS diagnosis from symptom onset ranging from 16 - 24.8 hours and in-hospital delays ranging from 9.6 -12.7 hours [94-97]. In one recent study of 209 children with acute AIS, although 58% of children reached hospital within 3 hours and another 12% within 3-6 hours, only 20% of children were diagnosed with stroke within 6 hours of symptom onset (10% within 3 hours). In children that had their symptom onset outside the hospital, the median interval from onset of symptoms to hospital arrival was 1.7 hours and from hospital arrival to initial neuroimaging was 3.1 hours and the AIS diagnosis was 12.9 hours (range 5 -31.1 hours) [96]. In this study, the total median delay was 29-hours (range 11.3 -65.9 hours). Additionally, ischemic stroke was not clinically suspected in over 62% of these children at initial presentation. This data indicate that much needs to be done to overcome the existing delays in pediatric stroke diagnosis. Prompt and correct etiological diagnosis in children with ischemic stroke is necessary not only for the appropriate and timely hyperacute therapeutic intervention but also for the prediction of recovery from stroke and the stroke recurrence rate [24, 25, 98].

Acute Antithrombotic Therapy

During the acute treatment phase, if not enrolled in the t-PA trials, treatment with an antithrombotic (either anticoagulant or antiplatelet) therapy is advised (a 2C or class I recommendation with level of evidence A). This recommendation is based on the adult literature and few pediatric case studies that have demonstrated both the safety and the efficacy of antithrombotic treatment in children with AIS [99-107]. A single prospective study compared the effects of Aspirin to low molecular weight heparin in 135 children with AIS and found no statistically significant differences in the two treatment groups Strater [107]. A very recent study by Schechter and colleagues [108] retrospectively assessed safety of anticoagulation among 215 children with AIS. In their study, 123 children received anticoagulants within 7 days of AIS diagnosis. The rate of new or increased intracerebral hemorrhage was 11% (symptomatic 4%, asymptomatic 7%) in children treated with anticoagulant therapy and 16% in the non-anticoagulant treated group. The hemorrhages were mild in all children treated with anticoagulant therapy, except in one child and all hemorrhages occurred within one month of the initiation of anticoagulant therapy [108].

However, lack of randomized controlled studies regarding the choice of antithrombotic agents (antiplatelets versus anticoagulants) in children with AIS has generated much controversy. This controversy is evident in the two simultaneously published guidelines outlining the management of ischemic stroke in children, the United Kingdom Royal College of physicians Pediatric Stroke Working group (UK) and the American College of Chest Physicians (ACCP) practice guidelines (this guideline was updated in year 2008). Both

guidelines were consensus or working group recommendations among physicians with expertise in pediatric stroke (including neurologists, hematologists, neurosurgeons and physiatrists and rehabilitation therapists). [44, 68-70, 109]. Subsequent to these two guidelines, American Heart Association (AHA) guidelines and the Canadian Medical Association (CMA) best practice recommendations were published for the management of stroke in infants and children. The AHA and ACCP guidelines had similar recommendations for the acute and preventative treatment of AIS in children [44, 109]. Both recommended acute treatment with anticoagulation (within 12-hours – 24 hours of stroke symptoms onset) until cardioembolic and craniocervical dissection etiologies are ruled out. However differences existed in the duration of anticoagulant therapy. According to the ACCP guideline, for children with confirmed AIS without hemorrhage, anticoagulation with standard unfractionated or low molecular weight heparin is recommended in the first 5 to 7 days until the evaluation for underlying etiologies and risk factors is completed. The dose of heparin is adjusted based on the serum anti Xa activity (with target anti Xa factor levels of 0.5-1.0 U/ml for standard unfractionated heparin and 0.35-0.7 U/ml for low molecular weight heparin). In situations where the coagulation system plays a major role, such as cardioembolic stroke, arterial dissection and prothrombotic states, children are advised to be continued on either oral (preferably warfarin with target therapeutic INR of 2.0-3.0) or subcutaneous anticoagulants for 3-6 months and then switched to an antiplatelet agent, usually aspirin. AHA recommended that continued treatment with anticoagulation is advised for at least six weeks or until cerebral arterial abnormalities resolve/improve or underlying heart defect is corrected, followed by switch to antiplatelet therapy. The UK guidelines had different recommendations and advised antiplatelet therapy with aspirin (3 – 5 mgs/kg/day) for both initial acute and preventative treatment of AIS in children [70]. However, they advised that anticoagulation may be considered and discussed with the senior pediatric cardiologist and hematologist for cardioembolic stroke and with pediatric hematologist for children with extracranial cervical dissection (without hemorrhage) and prothrombotic states [70]. The CMA best practice recommendations have primarily endorsed the AHA guidelines [68].

These guidelines clearly indicated the urgent need for systematic randomized studies for the treatment of children with AIS. Efforts are now underway among the international pediatric stroke network investigators to address these important questions and controversies.

Supportive Therapy

In children with AIS, until more research has been undertaken regarding efficacy and safety of various potential therapies that may be used during the hyperacute period, the general supportive measures are the mainstay in the management of these children.

The general supportive measures are aimed at decreasing the volume of infarction by salvaging the brain tissue that is at risk of permanent infarction (known as the penumbra). These recommendations are most comprehensively addressed by the AHA guidelines and include maintenance of adequate cerebral oxygenation and blood flow, peripheral hemodynamic stability by adequate hydration and avoidance of fluctuations in blood pressures, maintenance of normoglycemic states, prompt and aggressive treatment of seizures and fever (class I recommendation, level of evidence C), and prevention and treatment of raised intracranial pressure, such as decompressive hemicraniectomy for malignant hemispheric stroke, (class IIa recommendation, level of evidence B) [14, 44, 68].

Long Term Therapy

Long term or chronic therapy is aimed at prevention of recurrent stroke, stabilization and management of underlying etiologic disorder (if known) and stroke rehabilitation.

Secondary Prevention

The risk of recurrence for childhood AIS is as high as 15% -35 [11, 24, 25, 110-112]. After the initial acute phase, treatment is therefore targeted to the prevention of stroke in children. The secondary prevention therapies for adult AIS has been shown to significantly reduce risk of recurrence and hence improve outcome [113]. However, as for acute therapy, there is no evidence for secondary prevention in children with AIS. The preventative treatment approaches are again based on adult safety and efficacy data and the recently published pediatric stroke management. All the guidelines recommend secondary prevention with an antiplatelet agent in pediatric patients with AIS, except in children that have high risk for recurrent thromboembolism. These exceptions included certain cardioembolic conditions (usually until the defect is corrected), selected prothrombotic states, and conditions associated with recurrent and multiple thromboembolic events. In these high risk children, antithrombotic therapy with anticoagulants is advised (as described for the acute treatment phase). In children, the usual recommended antiplatelet agent is aspirin, at a dose of 3-5mg/kg/day, given in once a day dose. For children that have demonstrable resistance to aspirin, treatment with another antiplatelet agent is advised, such as clopidogrel [44]. Additionally, in children that have failed aspirin or have recurrent strokes on aspirin, a switch to anticoagulation was considered by UK [70] and either anticoagulation or another antiplatelet agent by ACCP [109]. This issue was not specifically addressed by the AHA guidelines [44].

It should be noted that the above recommendations only apply to childhood AIS beyond the neonatal age group. In children with ischemic perinatal stroke, the almost negligible risk of stroke recurrence and lack of safety data does not justify treatment with thrombolytic or antithrombotic agents. The decision to treat selected neonates with antithrombotic therapy under some circumstances is rare and dependent on the underlying condition and risk of recurrent thromboembolism. These treatment considerations should always be reviewed carefully with all treating physicians and the parents/caregivers.

Rehabilitative Approaches

As in adults, traditional rehabilitative therapies are generally considered to be beneficial in children with stroke in enhancing recovery and improving function, perhaps more effective in children owing to the amazing brain plasticity at this young age. In spite of the paucity of systemic studies for these rehabilitative approaches, there is general consensus that during the acute stroke phase, these assessments should be obtained to determine need for both acute and long term rehabilitative services including: physiotherapy, occupational therapy, swallowing assessment and speech and language therapy (if required) [44, 68, 70]. In children that have suffered stroke, psychological and cognitive assessments and processes to support their adequate placement or settlement in the school (development of an individual education plan, educational assistance, etc.) and the community are other important factors to consider in this regard. There are other non-traditional therapies that, either alone or in combination,

appear to have the potential to enhance motor outcome even years after the initial stroke. These include constraint induced therapy, transcranial magnetic stimulation and conductive motor learning [114-122]. To date, two randomized controlled studies have been performed with constraint induced therapy in children with hemiplegic cerebral palsy [114, 118, 120], and one with transcranial magnetic stimulation in children with AIS [123]. These studies have shown promising results and are further explored either alone or in combination for clinical application. In addition, tendon lengthening procedures and local Botulinum toxin A injections for spasticity have shown to improve functional outcome in children with hemiplegic cerebral palsy, when paired with other rehabilitative therapies [124-126].

Treatment of Specific Etiological Disorders Associated with Ischemic Stroke

In this section the clinical management of some specific childhood disorders and etiological conditions associated with stroke are discussed.

In children with cardioembolic disorders, if possible, immediate correction of the underlying congenital or acquired defect is advised. Similarly, for cardiac valvular disease, valve repair or replacement is indicated. For patients with atrial growth such as myxoma, removal of the tumor is urgently advised. Children with severe cardiomyopathies and poor ejection fraction may require cardiac transplantation [44].

Children with evidence of active infection will require treatment with appropriate antimicrobial or antiviral therapy whereas children with stroke due to either diffuse multisystem vasculitis or primary angitis of the central nervous system require immunosuppressant treatments such as systemic steroids, cyclophosphamide or azathioprine [44].

Based on the landmark randomized trial in children with sickle cell disease (SCD), the Stroke Prevention Trial in Sickle Cell Anemia (STOP), optimal hydration, periodic transfusions to reduce sickle hemoglobin and monitoring with transcranial doppler (TCD) study is recommended [44, 70, 109, 127]. The STOP study compared periodic blood transfusions to standard care in children ages 2 to 16 years that were identified to be at high risk of stroke with TCD monitoring [128]. The STOP trial found that the reduction of sickle hemoglobin to less than 30% reduces the risk of stroke from 10% to 1% per year. In all children with SCD (with or without stroke), annual monitoring with TCD is recommended for all normal studies, in 3-6 months for mildly abnormal studies and in one month for all significantly abnormal studies (Class IIa recommendation, Level of Evidence B). In children with at least two abnormal TCD studies, periodic transfusion to reduce sickle hemoglobin are recommended (Class I recommendation, level of evidence A). In children with sickle cell disease (SCD) and stroke due to sickle cell crisis, the recommendations are: 1) Optimal hydration with intravenous fluids and correction of hypoxemia and hypotension (Class I, level of evidence C), 2) Exchange transfusion with an aim to keep sickle hemoglobin (HbSS) less than 30% and hemoglobin between 10-12.5 g/dL (Class IIa, level of evidence C), 3) After the initial exchange transfusion, long term periodic blood transfusions to reduce sickle hemoglobin (Class I, level of evidence B) [44, 70, 109]. The guidelines also recommend considerations for hydroxyurea and bone marrow transplantation for children refractory to above therapies. The preventative treatment of SCD patients with stroke due to vasculopathy has not been systematically studied. In these children, as in other stroke cases due to an

arteriopathy, antiplatelet therapy may be beneficial for secondary prevention. In addition, revascularization surgery (especially in patients with secondary moyamoya) may be considered in refractory cases [44].

In children with moyamoya disease and syndrome, special precautions are advised to avoid alterations in cerebral blood flow and cerebral vasoconstriction such as maintenance of arterial PCO₂ over 25mmHG during situations requiring general anesthesia, avoiding prolonged hyperextension of neck and maintaining stable mean arterial pressures (Class IIb, Level of Evidence C) [44]. Revascularization surgery via either indirect method such as encephaloduroarteriosynangiosis (EDAS) or pial synangiosis or direct method such as superficial temporal artery to middle cerebral artery by-pass should be considered in all patients with moyamoya (Class I, Level of Evidence B). Due to the presence of small arterial vasculature in young children, indirect methods are the preferred revascularization procedures in children with moyamoya. For both symptomatic and asymptomatic moyamoya patients, with or without revascularization surgery, preventative therapy with antiplatelet therapy is advised, except in patients with recent hemorrhage or bleeding tendencies (Class IIb, Level of Evidence C) [44].

In children with a genetic disorder, efforts should be made to treat the underlying biochemical defect (Class I, Level of Evidence B). For example, in patients with hyperhomocysteinemia, cofactor therapy with vitamin B12, folic acid and vitamin B6 is advised and in patients with Fabry disease, enzyme replacement therapy is now available and should be considered in children with recurrent stroke [44].

RECOVERY AND OUTCOME

In children with AIS, permanent motor or cognitive disabilities are observed in at least 60% to 75% of stroke survivors [16, 129-131]. Hemiparesis is the most commonly reported residual neurological deficit and is present in at least 60% of children. Other long term neurological deficits include bilateral or other motor difficulties in 11% , cognitive and behavioral deficits in 11% and speech and language difficulties in 22% of children with AIS [132]. Cognitive, memory and behavioral deficits, although commonly reported in young children, may not be as disabling at younger ages and only become apparent later [133-135]. Additional long term sequelae that can be quite disabling include structural epilepsy, movement disorders and headache. These are observed in about 15%-30% of children with AIS [6, 17, 132, 136, 137]. These permanent long term deficits significantly contribute in reducing the quality of life in survivors of childhood AIS, which also affects the entire family dynamics and the community at large [110, 138]. Mortality from stroke is reported to occur in 5%–28% [111, 130, 132]. In childhood AIS, death is mostly due to large hemispheric stroke with massive cerebral edema resulting in transtentorial or posterior fossa herniation.

The indicators of poor motor and cognitive outcome in children with AIS include young age at the time of stroke (< 12 months), alteration in consciousness and/or seizures at presentation, cortical middle cerebral artery territory infarction (especially large infarcts that occupy >10% of intracranial volume), internal capsule infarction and the presence of certain prothrombotic abnormalities [57, 111, 139]. In addition, in children the AIS outcome is also dependent on the associated etiopathogenetic conditions and co morbid disorders [25, 39,

140]. However, it is generally believed that children with brain injury have comparatively better outcomes than adults, mainly due to brain plasticity and ability of the cortex to functionally reorganize at this young age.

Recurrent ischemic events are reported in 20%-37% of children with AIS, with the highest risk reported in the first year after initial stroke [5, 25, 39, 112, 132, 141]. It should be noted that the secondary prevention strategies used in these studies varied widely and may have included children that may not have received any prophylaxis, especially in older studies. If no prophylactic therapy is received, the rate of recurrent ischemic events may be as high as 50% [112]. The factors associated with increased recurrent rates include arteriopathies with persistent vascular abnormalities [25, 39, 140, 141], presence of certain hypercoagulable states (such as MTHFR T677T, elevated Lp(a), hyperhomocysteinemia, protein C deficiency and prothrombin gene mutation) [98, 142] and coexistence of multiple risk factors in the same individual child [25, 112].

CEREBRAL SINOVENOUS THROMBOSIS

Cerebral sinovenous thrombosis is characterized by the presence of thrombus or flow interruption within the cerebral dural venous sinuses or veins which may or may not be associated with ischemic or hemorrhagic infarction. The thrombosis may involve the superficial venous system (the superior sagittal sinus and the lateral sinus), deep venous system (the internal cerebral veins, vein of Galen, and the straight sinus), or both deep and superficial systems.

In comparison to arterial ischemic stroke, cerebral sinovenous thrombosis is an uncommon form of stroke, representing 0.5% to 1% of all strokes. In adults, the incidence of CSVT has not been reported among any population studies. The data is limited to few cohort studies and stroke registries that included CSVT patients. The largest cohort study is the International Study on Cerebral Venous and Dural Sinuses Thrombosis [ISCVT], which demonstrated that 487 (78%) of 624 CSVT cases occurred in patients younger than 50 years of age. The Mexican stroke registry noted that 3% of all stroke cases were CSVT. A clinic-based stroke registry in Iran reported an annual CSVT incidence of 12.3 per million [143]. In children, the CSVT literature includes single case reports and series and a single landmark population based study, the Canadian Pediatric Ischemic Stroke Registry (CPISR) [144-149]. In the CPISR, the incidence of CSVT was estimated as 0.67 per 100,000 children per year in Canada. The CPISR studied 160 children (newborn - 18 years of age) with clinical symptoms and radiographic confirmation of cerebral sinovenous thrombosis and noted that over 40% of the CSVT cases occurred during the neonatal period (0-28 days of life). Over 55% of children presented without ischemic infarction and in 28% hemorrhage, parenchymal and or intraventricular was present in children with CSVT [144].

CLINICAL PRESENTATION

The clinical presentation of CSVT in children includes headache, altered level of consciousness, seizures, irritability and focal neurological deficits. As in adults, the degree

and type of neurological dysfunction in CSVT is related to the location of thrombosis and the presence of hemorrhage or ischemic infarction and an increase in intracranial pressure due to impaired venous drainage. Often at the time of initial presentation or with progression of CSVT, patients show clinical findings pertaining to both infarction and raised intracranial pressure. Common symptoms for CSVT in children in decreasing order of frequency included seizures in 58%, decreased level of consciousness in 44%, focal neurological deficits in 42%, headache due to increase in intracranial pressure in 34% (59% of non-neonates), and irritability/jitteriness in 17% (mainly infants) [144]. Headache in CSVT is described as diffuse and progresses in severity. In older children, the different types of headaches include the thunderclap headache (indicative of subarachnoid hemorrhage), migrainous headache, and isolated non-specific headache without focal neurological findings or papilledema. Older children that present with the typical symptoms of headache, papilledema, and or diplopia (caused by sixth nerve palsy) due intracranial hypertension, even without other neurological focal signs, should be probed for the CSVT diagnosis.

With the regards to the location of thrombosis, the Canadian Registry notes that 86% of thrombosis is located in the superficial venous system and 38% in the deep venous system [144]. Thrombosis in the superior sagittal sinus commonly leads to headache, increased intracranial pressure and papilledema. Older children with bihemispheric venous infarction and or hemorrhage due to superior sagittal sinus thrombosis may present with paraparesis. In addition, other focal motor deficit, sometimes with seizures, may occur as well as scalp edema and dilated scalp veins that can be seen on examination. For lateral sinus thrombosis which is often associated with middle ear infection, symptoms such as fever, ear discharge, pain in the ear or mastoid region and headache are common. The deep cerebral venous system thrombosis results in thalamic or basal ganglia infarction. Most children with deep CSVT present with rapid neurological deterioration, mainly decreased level of consciousness. Cortical vein thrombosis although not uncommon but clinical syndromes (e.g., temporal lobe hemorrhage associated with vein of Labbe' thrombosis) associated to the larger cortical vein thrombosis are infrequently seen in children.

ETIOLOGIES AND RISK FACTORS

Various risk factors contribute to the etiology of childhood CSVT. Traditionally, etiologies for CSVT can be categorized as either 'infectious' or 'non-infectious'. The infectious CSVT, accounts for 18% of pediatric CSVT and is identified by thrombosis of venous sinuses due to infections in the head and neck or systemic infection (for example: otitis media, mastoiditis or sinusitis, sepsis). In children, infection-related CSVT is very common as oppose to adults. In comparison, the mechanism that initiates thrombosis in non-infectious CSVT has yet to be properly determined. Underlying risk factors for non-infectious CSVT can be divided into two groups. This first group being acquired risk factors which include surgery, head trauma, dehydration, congenital heart disease, chronic systemic diseases such as inflammatory bowel disease, nephrotic syndrome, Behçet disease, iron-deficiency anemia, exogenous hormones or procoagulant drugs (e.g., L- Asparaginase, oral contraceptives) or states (e.g., cancer). The correlation of cancer, commonly hematologic malignancies, to childhood CSVT has been speculated for some time. The CPISR has

reported that 8% of cases of childhood CSVT have some type of cancer. The likely mechanisms associated with cancer in children with CSVT include direct tumor compression, tumor invasion of cerebral sinuses, and the hypercoagulable states associated with cancer and chemotherapeutic and hormonal agents used for cancer treatment. Other uncommon causes that have been related with CSVT include: paroxysmal nocturnal hemoglobinuria, thrombocythemia, heparin-induced thrombocytopenia, thrombotic thrombocytopenic purpura [143]. The second is genetic risk factors or inherited thrombophilia such as deficiency of antithrombin III, protein C, and protein S; mutations in factor V Leiden, prothrombin gene and hyperhomocysteinemia [143, 144, 150-153] (Table 3).

In comparison to adults, prothrombotic disorders have been reported to occur in 20% - 80% of childhood CSVT cases. [143, 144, 151-153]. Most helpful is the recent meta-analysis by Kenet and colleagues [154] which [154] investigated both inherited and acquired thrombophilic disorders in children (0 to 18 years of age) that had suffered an initial ischemic stroke event of any type (i.e., both ischemic stroke sub-types: arterial ischemic stroke (AIS) and cerebral sinovenous thrombosis (CSVT)). Twenty-two studies met inclusion criteria, with a total 1764 patients (1526 AIS, 238 CSVT) and 2799 control subjects were enrolled. They found that for each thrombophilia trait investigated a statistically significant association with first stroke existed. In addition, they found no difference between AIS and CSVT patients. The summary odds ratios for each trait investigated included : antithrombin deficiency, 7.06 (95% CI, 2.44 to 22.42); protein C deficiency, 8.76 (95% CI, 4.53 to 16.96); protein S deficiency, 3.20 (95% CI, 1.22 to 8.40), factor V G1691A, 3.26 (95% CI, 2.59 to 4.10); factor II G20210A, 2.43 (95% CI, 1.67 to 3.51); 6.95 (95% CI, 3.67 to 13.14), and combined thrombophilias, 11.86 (95% CI, 5.93 to 23.73).

CLINICAL DIAGNOSIS

The clinical findings suggestive of CSVT and the specific imaging methods are necessary for diagnosing CSVT accurately and promptly. The slow progression and later appearance of symptoms often complicates and delays CSVT diagnosis and treatment. Presence of various risk factors and symptoms for CSVT further contribute to these challenges. Several important clinical features that contribute in identifying CSVT from other cerebrovascular abnormalities include focal or generalized seizures, changes in the level of consciousness without focal neurological findings, objective signs of raised intracranial pressure and bilateral brain involvement. In recent years, the widespread availability of neuroimaging tests, in particular magnetic resonance imaging (MRI), has allowed improved detection and diagnosis of ischemic stroke in children. The diagnosis of CSVT requires neuroimaging evidence of thrombus or lack of flow in the cerebral veins or venous sinuses either by head CT venogram (CTV) or MR venogram (MRV) with contrast (Figure 5). Head CT alone may miss the presence of infarct and/or sinus involvement. A non-contrast CT or MR venogram can often lead to the erroneous diagnosis of CSVT. A false positive result on non-contrast imaging may be due to the increased hematocrit in neonates and most commonly low blood flow in the cerebral venous system. In the CPISR, initial non-contrast head CT scan missed the diagnosis of CSVT in over 15% of patients. The characteristic findings that suggest CSVT on a non-contrast CT include 'dense triangle' or the 'cord sign' which indicates hyperdense thrombus in

the sinovenous channels. On the contrast enhanced venogram, an empty triangle or the ‘delta sign’ represents a clot in the venous sinuses. Combined with ability to detect early parenchymal lesions by diffusion weighted sequences and absent flow and presence of thrombus via venogram without radiation effects, brain MRI and MR venogram have become the diagnostic imaging modalities for CSVT at many paediatric hospitals [143].

Table 3. Etiologies and Risk factors for Cerebral Sinovenous Thrombosis in Children

Systemic Risk Factors	Prothrombotic Disorders and States
Dehydration	<i>Inherited Causes</i>
Septicemia	Protein C deficiency
Fever	Protein S deficiency
Malignancy	Activated Protein C resistance
Head and neck Disorders	Lupus anticoagulant presence
Head and neck infections(e.g., otitis media, mastoiditis, sinusitis, local abscess, meningitis)	Antiphospholipid antibodies presence
upper respiratory tract infections	Hyperhomocystenemia
Local compression of venous sinuses	Factor V Leiden mutation
Tumor invasion of venous sinuses	Prothrombin 20210A mutation
Head and neck Injury	<i>Acquired Causes</i>
Cranial Surgery	Cancer
Hematological Disorders	Procoagulant drugs (e.g., L-asparaginase, steroids)
Hemoglobinopathy (e.g., sickle cell disease)	Nephrotic syndrome
Iron deficiency anemia	Cyanotic congenital or acquired heart disease
Paroxysmal nocturnal hemoglobinuria	Autoimmune Disorders
Polycythemia	Systemic lupus erythematosus
Thrombocythemia	Sarcoidosis
Thrombotic thrombocytopenic purpura	Inflammatory bowel disease
Heparin-induced thrombocytopenia	Behçet disease
	Thyroid disorders

TREATMENT

As with AIS, the treatment of CSVT includes both general supportive measures and antithrombotic therapy. In addition, treatment of underlying etiology is extremely important such as treatment of infections and dehydration. The antithrombotic therapy is aimed at preventing the clot propagation and recurrence within the cerebral venous system. For children (outside the neonatal age group) without evidence of significant intracranial hemorrhage, both ACCP and AHA guidelines recommend treatment with anticoagulation for duration 3-6 months, with reassessment of re-canalization at 3-months. With significant intracranial hemorrhage, monitoring with serial neuroimaging is advised and in case of clot propagation in these patients, treatment with anticoagulation is advised [109, 143]. However, in neonates, there is no consensus. The ACCP guidelines recommend treatment with anticoagulation in neonates without large ischemic infarction or intracerebral hemorrhage and

radiographic monitoring for children with intracerebral hemorrhage with the recommendation to initiate anticoagulation if the extension of thrombosis occurs [109]. The AHA recommendations are to provide supportive therapy such as treatment of dehydration, infection, raised intracranial pressure and seizures. The AHA recommends that anticoagulation may be considered in selected neonates that show clinical or radiological propagation CSVT in spite of supportive therapy [143].

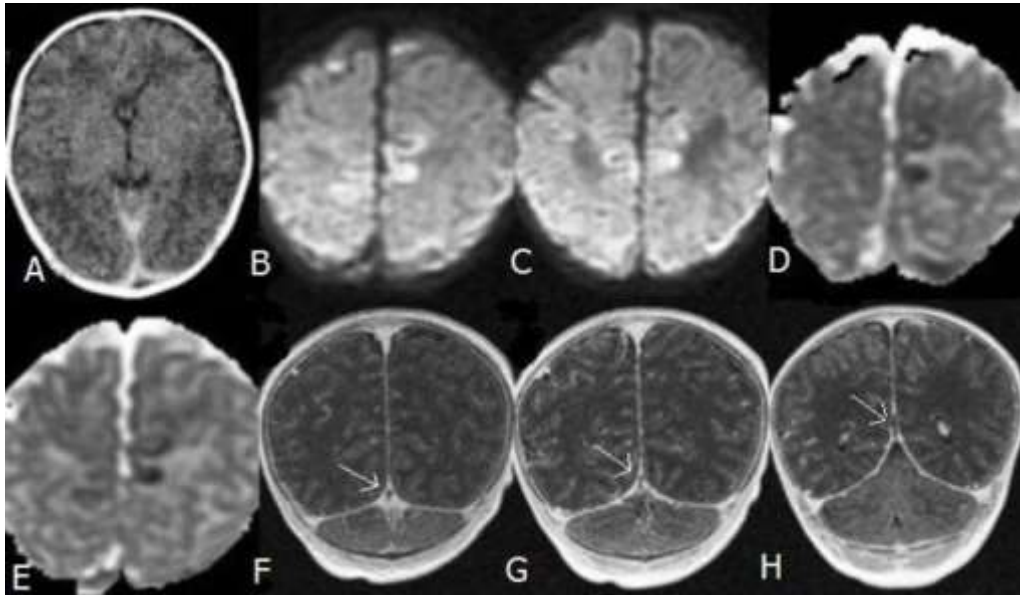


Figure 5. A term infant with cerebral sinovenous thrombosis who presented at the age of 20 days with decreased feeding, lethargy and generalized seizures. A) Initial CT head demonstrating evidence of poor grey and white matter differentiation, sulcal effacement and small ventricles consistent with diffuse cerebral edema and presence of hyperintensity along the falx consistent with blood in the subarachnoid space. B and C) A subsequent MRI brain showing evidence of bilateral diffusion restriction on the diffusion weighted images with corresponding apparent diffusion coefficient map consistent with bilateral paramedian frontal lobe ischemic infarction. D, E and F) The gadolinium enhanced MR venogram demonstrating presence of filling defect in the straight and the superior sagittal dural venous sinuses confirming thrombosis in these venous sinuses (arrows).

FUTURE DIRECTIONS

Research into childhood stroke is at early stages. Almost all management approaches are extrapolated from adult data. The International Pediatric Stroke Study (IPSS) (<https://app3.ccb.sickkids.ca/cstrokestudy>) and its network of investigators are making remarkable but early efforts to improve stroke care in children. Some of these great efforts includes identifying role of infection in pediatric cerebral vasculopathies and determining safety of hyperacute t-PA therapy in children (which is currently standard of care in adults). This global and multicentre participation has and will continue to facilitate systematic research in pediatric stroke thereby enhancing our overall understanding of this disabling disorder in children.

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Chapter 11

THE IMMUNE SYSTEM AND STROKES

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ABSTRACT

The immune system is an incredible defense mechanism which protects us against a myriad of potential pathogens like viruses, bacteria, protozoa, etc. It often shows rapid, very protective and highly specific responses. When the immune system is activated, a series of complex and inter-dependent cellular responses occur. An incredible feature of the immune system is to discriminate self from non-self. This skill of the immune system is extremely essential to destroy invading pathogens and altered cancer cells. Our first line of defense against foreign organisms are barrier tissues such as the skin that stops the entry of organisms into our body. If, however, these barriers are penetrated, the body contains cells that respond rapidly to the invaders. The cells of the immune system interact with one another by a variety of signal molecules so that a coordinated response may be mounted. These signals may be secretory molecules such as lymphokines, cytokines and chemokine's or receptors and their ligands on the cell surface which stimulate cells of the immune system. Although these two arms (innate and adaptive) of the immune system have distinct functions, there is interplay between them to influence each other. Although for many years the CNS was considered an immune-privileged organ, it is now well accepted that the immune system and the nervous system are engaged in bidirectional crosstalk. Moreover, mounting data suggests that in the brain, as in peripheral organs, inflammatory cells participate in tissue remodeling after injury. Microglial cells are the resident macrophages of the brain and play a critical role as resident immunocompetent and phagocytic cells in the CNS. Stroke is the second most common cause of death worldwide and a major cause of acquired disability in adults. Despite tremendous progress in understanding the pathophysiology of stroke, the translation of this knowledge into effective therapies has largely failed, with the

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exception of thrombolysis, which only benefits a small proportion of patients. Systemic and local immune responses have important roles in causing a stroke. However, potential therapeutic targets in the immune system and inflammatory responses have not been well characterized. Development of novel and effective therapeutic strategies for a stroke will require further investigation of these pathways in terms of their temporal profile (before, during, and after the stroke) and risk-to-benefit therapeutic ratio of modulating them.

INTRODUCTION

Systemic and local immune responses have important roles in causing stroke and are implicated in the primary and secondary progression of ischemic lesions, as well as in repair, recovery, and overall outcome after a stroke. In this chapter, we will discuss the effects of innate and adaptive immune responses in the CNS and overall outcomes in patients with stroke, and discuss their contribution to stroke onset, ischemic brain damage, and recovery. We know that all cells of the immune system have their origin in the bone marrow and they include myeloid and lymphoid which differentiate along distinct pathways. The myeloid progenitor cell in the bone marrow gives rise to erythrocytes, platelets, neutrophils, monocytes/macrophages and dendritic cells (DCs), whereas the lymphoid progenitor cell gives rise to the natural killer (NK) cells, T cells and B cells. For T cell development, the precursor T cell must migrate to the thymus where they undergo differentiation into two distinct types, the CD4⁺ helper T cell and the CD8⁺ cytotoxic T cell. CD4⁺ helper T cells can be further subdivided on the basis of the cytokine profile into Th1 and Th2 cells. Th1 cells secrete mainly IL-2, IFN- γ , lymphotoxin, etc. and are responsible for cell mediated immunity. In contrast Th2 cells produce chiefly IL-4, IL-5 and IL-10 and are responsible for humoral immunity (Mossman et al. 1989). B cells originate from bone marrow in mouse, human and other mammals and from bursa of Fabricius in birds. B cells produce diverse repertoires of antibodies, synonymously called immunoglobulins (Igs).

CELLS OF THE IMMUNE SYSTEM

The immune system consists of a wide range of distinct cells, each with a specific role to play. The myeloid lineage of cells includes macrophages, dendritic cells, monocytes, mast cells and basophils. The lymphoid lineage includes NK, B cells and T cells. Some of these cells like B cells, macrophages and dendritic cells help in antigen presentation to T cells. The cells of the immune system are found in peripheral organized tissues like spleen, lymph nodes, Payer's patches of the intestine and tonsils etc.

The immune responses occur in these sites. The cells of lymphoid origin are also found in central lymphoid organs like the thymus and bone marrow where they undergo maturation. Lymphocytes and macrophages constitute a substantial portion of recirculating pool of cells found in the blood and lymph and provide immunity at the sites where it is needed. Some specialized cells like epithelial and stromal cells provide the anatomic environment in which immunity occurs by secreting many chemical factors that regulate growth and gene activation

in cells of the immune system. They also play a direct role in the induction and effector phase of the immune response.

The response to pathogens is orchestrated by the complex interactions and activities of the large number of diverse cell types involved in the immune response. The innate immune response is the first line of defense and occurs soon after pathogen exposure. It is carried out by phagocytic cells such as neutrophils and macrophages, NK cells, and granulocytes (Medzhitov et. al. 2001, 1997). The subsequent adaptive immune response includes antigen-specific defense mechanisms and may take days to develop. Cell types with critical roles in adaptive immunity are T cells and B cells.

Antigen-presenting cells (APCs) are critical players in the immune response. The function of these cells is to engulf pathogens or dying cells, process them and finally present their antigenic epitopes in context with MHC molecules to T cells. However, DCs are the only cells involved in antigen presentation to the naïve T cells. For optimum activation of naïve T cells, two signals are required. The first signal is antigen specific and is delivered through binding of the T-cell receptor (TCR) to peptide/MHC complexes on the APC surface. The second signal is non-specific and is also APC driven in the form of costimulatory molecules (Lenschow et. al. 1996). Among APCs only DCs constitutively express these costimulatory molecules. Several other cells, classified as non-professional APC, can be induced to express MHC-II molecules or costimulatory signals to work as an APC. Many of these cells like fibroblasts, astrocytes, epithelial cells, etc., function as an APC only for a short period during a sustained inflammatory response (Sung et. al. 1997, Pechhold et. al. 1997). Moreover, the main professional APCs are dendritic cells, macrophages and B cells.

Strokes are the second most common cause of death worldwide and a major cause of acquired disability in adults. Despite tremendous progress in understanding the pathophysiology of a stroke, translation of this knowledge into effective therapies has largely failed, with the exception of thrombolysis, which only benefits a small proportion of patients. Systemic and local immune responses have important roles in causing strokes and are implicated in the primary and secondary progression of ischemic lesions, as well as in repair, recovery, and overall outcome after a stroke. However, potential therapeutic targets in the immune system and inflammatory responses have not been well characterized. Development of novel and effective therapeutic strategies for strokes will require further investigation of these pathways in terms of their temporal profile (before, during, and after the stroke) and risk-to-benefit therapeutic ratio of modulating them.

Worldwide, around 15 million people have a stroke every year, and with about 5 million deaths, strokes are the second most common cause of death and a major cause of long-term disability [AHA 2010]. It is estimated that about 25% of people older than 85 years will develop a stroke. In the past 10 years, remarkable progress in understanding stroke pathophysiology has been made, especially in ischemic stroke, which makes up 80% of all cases. These advances have led to the identification of more than 1000 molecules with brain-protective effects from experimental models and to the implementation of more than 250 clinical trials. Despite all these efforts, translation into effective therapies has failed. Reperfusion induced by alteplase (tissue plasminogen activator; TPA) is the only acute pharmacological treatment approved by the health authorities [Young AR 2007, NIHD 1995]. Systemic inflammation is strongly linked to the occurrence of a stroke, and inflammation might have deleterious effects during and after an event, triggering various cascades of damage. However, some recent data show that inflammatory processes might also have

beneficial effects (figure 1.1). Such inflammatory responses involve not only peripheral cells such as leukocytes, but also brain cells such as glial cells, endothelial cells, and neurons. In this chapter, we will focus on the effects of innate and adaptive immune responses in the CNS and overall outcomes in stroke patients, and discuss their contribution to stroke onset, ischemic brain damage, and recovery. We also discuss potential immune-based stroke therapies. We postulate that a better understanding of the interactions between the immune system and the CNS will foster the development of innovative therapeutic strategies for patients with stroke.

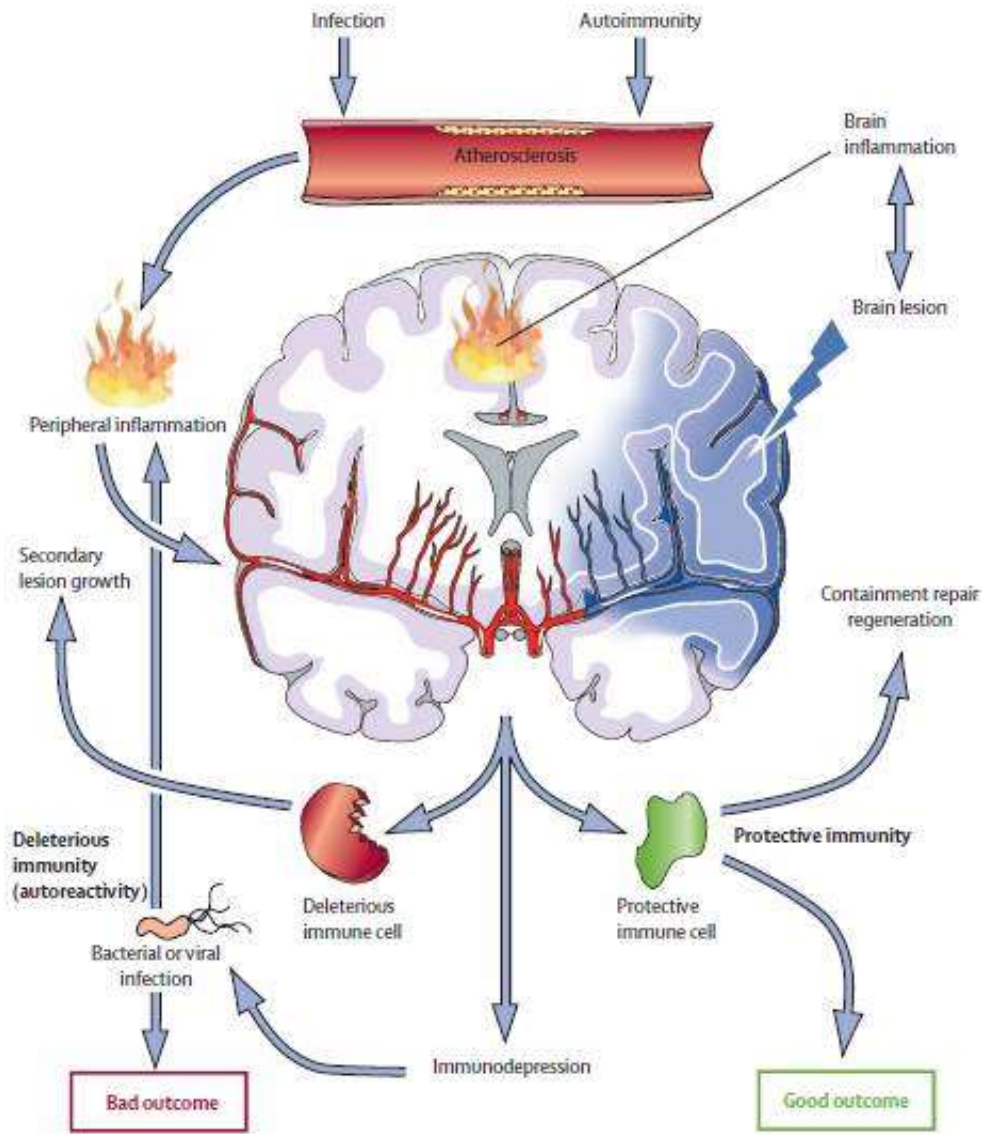


Figure 1.1. Complex interactions between peripheral inflammation (which might underlie the occurrence or atherosclerosis of a stroke), stroke-induced brain inflammation, and responses of the peripheral innate and adaptive immune systems to a stroke (inflammation, immunodepression, autoreactivity, or protective immunity).

Role of Inflammation and the Immune System in Stroke

Nearly all types of infectious agents have been suggested to increase incidence of a stroke. However, the risk of a stroke might be associated with an aggregate burden rather than with an individual pathogen. Moreover, a range of host factors, including genetic ones, probably interact with infectious or inflammatory conditions to influence the risk of vascular disease. Inflammatory processes affect stroke incidence through several mechanisms, including thrombosis through the coagulation system, vasculopathy through vasculitis or altered vascular reactivity, and, most often, by atherosclerosis (figure 1.2). The prothrombotic state reported during inflammation results from the activation of many cellular targets (e. g, monocytes, macrophages, platelets, endothelial cells, and T lymphocytes), leading to an imbalance between anticoagulant and procoagulant molecules, an increased production of chemokine's such as monocyte chemoattractant protein-1), cytokines (interleukin 1 β , 4, 6, and 10, tumor necrosis factor [TNF], interferon's and an altered production of adhesion molecules (P-selectin, E-selectin, L-selectin, vascular cell adhesion molecule 1 [VCAM1], intercellular adhesion molecule 1 [ICAM1], and integrin's such as CD11a-c). Circulating cells are thus recruited to the endothelial wall and promote local inflammatory events. Besides vasculitis, inflammation can also induce various processes in the cerebral microvasculature, such as blood-brain barrier leakage (partly mediated by matrix metalloproteinase [MMPs]), sequestration or adhesion of red blood cells on endothelial cells (leading to apoptosis of the latter), and platelet activation or aggregation. Among the recruited leukocytes, neutrophils, natural killer cells, dendritic cells, and macrophages produce mediators that further activate or even damage endothelial cells through activation of nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) pathways. Together, these events favor a T-helper-type-1 (Th1) environment for infiltrating T lymphocytes and induce their activation. Conversely, some experimental studies suggest that preinfections might have a protective role by increasing neurogenesis, promoting anti-inflammatory responses, inhibiting macrophage and T-cell accumulation within atherosclerotic lesions, and producing neuroprotective molecules and free radical scavengers, leading to a form of preconditioning. However, the consensus at present is that the cumulative effect of previous inflammation on stroke incidence is negative and suggests that inflammation is an important therapeutic target for stroke prevention. Thromboembolism is the most common mechanism of cerebrovascular occlusion, but a stroke can also manifest as a complication of systemic inflammatory disorders or autoimmune processes such as inflammatory bowel disease, primary or secondary vasculitis, rheumatoid arthritis, or antiphospholipid syndrome [Deb P 2010]. Compelling evidence links inflammation (acute or chronic) and risk of a stroke to systemic infection; for example, about 30% of patients with ischemic stroke present with a recent antecedent infection and stroke incidence increases with epidemics such as influenza pandemics.

Immune System-Brain Interactions in the Acute Phase of a Stroke

An ischemic stroke results in an abrupt deprivation of nutrient supplies that quickly leads to irreversible damage in the core of the affected area. Secondary processes, such as the partly excitotoxicity, oxidative stress, and mitochondrial disturbances, spread cellular damage into preserving peri-infarct area (penumbra). Ischemic reperfusion injury also induces a robust

inflammatory response that might be necessary for tissue repair and regeneration in advanced stages. Minutes to hours after onset of cerebral ischemia, a cascade of inflammatory events is initiated through the activation of resident cells (including microglia) and recruitment of circulating leukocytes. Primary signals that trigger upregulation of inflammatory mediators are endogenous molecules (damage-associated molecular patterns [DAMPs]) released from dying cells, such as high-mobility group box 1 (HMGB1), hyaluronan, or heat shock proteins [Chen GY 2010]. Many DAMPs are sensed by Toll-like receptors (TLRs), a receptor family with broad cellular expression originally described as microbial sensors of the innate immune system.

Studies of TLR-2-deficient or TLR-4-deficient mice reported that these TLRs have a harmful effect through increasing stroke-induced lesions and inflammation. Recently, a study [Lahnardt S 2010] showed that TLR-2 and TLR-1 require the recruitment of CD36 as a co-receptor to promote the inflammatory responses and tissue damage evoked by cerebral ischemia in rodents. TLR-2 may also be a therapeutic target to prevent atherosclerosis, since these receptors mediated inflammation in an in-vitro model of human atherosclerosis [Monaco C 2009]. However, agonists of TLR can induce protective preconditioning [Marsh BJ 2010, Leung PY 2010]. Among the most studied DAMPs is HMGB1—a ligand of TLR-2, TLR-4, and advanced glycation end product (RAGE) —which is released by damaged cells and exerts a deleterious proinflammatory effect in ischemic rodents. However, HMGB1 could have a biphasic action, with an acute inflammatory noxious effect on the early stage, followed by a promotion of repair processes later on, including neurogenesis and angiogenesis; this delayed beneficial response is associated with HMGB1-positive reactive astrocytes [Hayakawa K 2010a and 2010b]. Strategies that interfere with DAMP-receptor pathways might be a promising approach to restrict inflammation processes. Downstream of DAMP-receptor signaling, inflammatory mediators such as cytokines (interleukin-1 β , TNF, and interleukin-6), chemokines, nitric oxide, and reactive oxygen species are released, exacerbating cell death and eventually leading to dysfunction of the blood–brain barrier. Cytokines and chemokines induce upregulation of adhesion molecules, including P-selectin, E-selectin, and ICAM1 on the vascular endothelium, attracting and homing circulating leukocytes into the cerebral parenchyma.

Immune system preserves tissue homeostasis of the host when threatened by a pathogen or sterile injury (e.g., ischemia) via two mechanisms: innate (non-specific) immunity and adaptive immunity. An immune response that begins immediately after injury or infection and relies on various cell types including polynuclear cells, macrophages, and dendritic cells, as well as resident cells from tissue lesions such as endothelial cells or microglia. These cells recognize danger signals (damage-associated molecular patterns [DAMPs]) released upon tissue injury. Engagement of broad-specificity receptors such as Toll-like receptors by DAMPs triggers an inflammatory response marked by the release of proinflammatory cytokines and chemokines and recruitment of innate immune cells. An antigen-specific immune response depends on lymphocytes that have antigen-specific receptors. Unlike innate immunity, cells of adaptive immunity develop memory against the stimulating antigen. Full activation of immune cells needs two signals the main TCR signal and the other costimulatory signal. Responses are regulated by several subsets of CD4 T-helper (Th) cells characterized by their cytokine secretion. Th1 cells produce interferon- γ , which is involved in macrophage activation and leads to cell-mediated immunity, whereas Th2 cells release interleukin-4, which stimulates the production of antibodies by B cells. Interleukin-17-

producing T cells (Th17 cells) are a newly described subset implicated in the inflammatory response. The simplistic view of Th1–Th2 dichotomy is far from the true complexity in human diseases because Th polarization is relatively easy to define in murine models, whereas clear distinctions between the patterns are more difficult to establish in human beings. Innate T cells with a T-cell receptor that is distinct from that of conventional $\alpha\beta$ T cells. $\gamma\delta$ T cells do not recognize antigens displayed on MHC molecules, but rather recognize specific ligands expressed on stressed or infected cells but not healthy cells.

A physiological process that aims to limit tissue damage or infection, removes dead cells and debris, and promotes tissue remodeling and regeneration (irrespective of the initiating stimulus). However, inflammation might have adverse effects through collateral cellular damage, in particular in the brain parenchyma after a stroke. Some cells in the nervous system express MHC molecules and can thus act as antigen-presenting cells to regulate T cells. Notably, neurons that do not express MHC molecules can control the function of T cells.

Natural Regulatory T Cells

These cells express CD4, CD25, and the transcription factor forkhead box P3 (Foxp3) and are involved in the maintenance of peripheral tolerance. These cells act by cell-to-cell contact and by the release of immunosuppressive cytokines such as interleukin-10 or transforming growth factor- β . Other subsets of regulatory cells have also been described (e.g., CD8 T cells, B cells, or myeloid-derived suppressive cells). A peripheral tolerance mechanism induced by exposure to antigens at mucosal surfaces. The kinetics of recruitment of the different inflammatory cells are under debate, but it is accepted that neutrophils lead the migration into the brain parenchyma, followed by macrophages and lymphocytes a few days after injuring [Jin R 2010]. Additionally, dendritic cells might accumulate, but the role of these key inflammatory cells in ischemic remains to be established [Gelderblom M 2009]. The lymphocytes have a crucial function in inflammatory processes and are strongly associated with deleterious effects in strokes, as noted in T-cell and B-cell deficient mice, which have a strikingly reduced infarct size after transient focal ischemia [Hurn PD 2007]. Administration of the immunosuppressive drug fingolimod, which, among other effects, inhibits T-cell infiltration into inflammatory tissues, also reduced infarct volumes in a mouse model of ischemia [Schichita T 2009]. Progress has been made in understanding the role of different T-cell subsets in ischemic inflammation [Lo EH 2009]. Shichita and colleagues [Schichita T 2009] reported that a late infiltration of $\gamma\delta$ T cells was associated with the neurological injury. Their experiments with interleukin-17 or interleukin-23 knockout mice show that interleukin-17 produced by these $\gamma\delta$ T cells was involved in the pathogenic mechanism and its expression was dependent on interleukin-23, which is a cytokine mainly secreted by blood-derived macrophages when they infiltrate the brain. In mice, depletion of CD4/CD25/FOXP3-positive regulatory T cells with a specific anti-CD25 monoclonal antibody increases the size of the ischemic lesion and upregulates proinflammatory cytokines such as TNF, interleukin-1 β , and interferon. The neuroprotective effects of regulatory T cells were mediated by interleukin-10. Conversely, Ren and colleagues [Ren X 2010] could not confirm that regulatory T cells were involved in restricting the area of brain ischemic injury. Hence, the role of regulatory T cells in a stroke needs to be clarified. Besides infiltrating leukocytes, neurons, astrocytes, and microglia also participate in ischemic brain injury,

through the release of proinflammatory mediators [McCoy 2008, Didier N 2003, Hayakawa K 2008]. Under inflammatory conditions, however, activated microglia can convert to a macrophagic phenotype and thus also exert a neuroprotective info once by phagocytosis of necrotic cells and secretion of neurotrophic factors such as brain-derived neutrophic factor or insulin-like growth factor [Thored P 2009, Dennes A 2007]

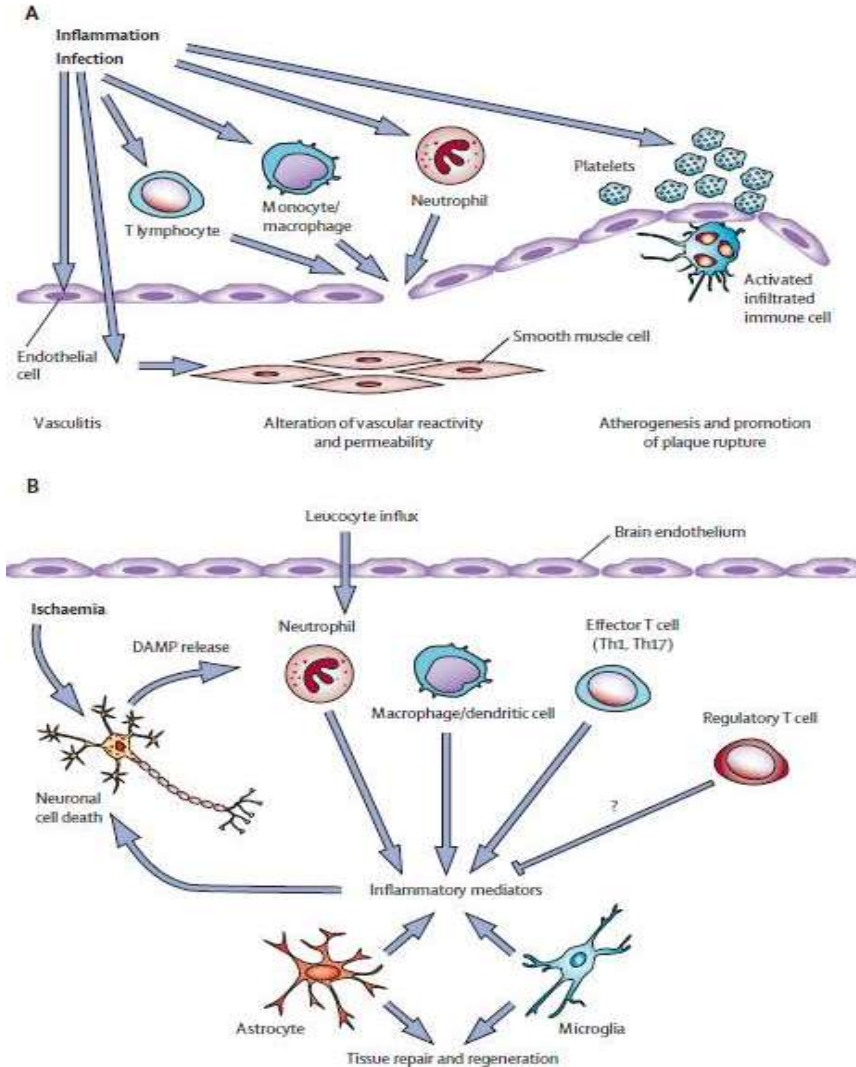


Figure 1.2. Inflammatory mechanisms before and after a stroke. (A) Inflammatory mechanisms that promote strokes: infection and inflammatory disorders can contribute to trigger cerebral ischaemia through pathophysiological processes such as vasculitis, changes of vascular reactivity, and especially atherosclerosis. (B) The acute phase of inflammation after a stroke: ischemia causes cell death in the brain parenchyma and subsequent release of endogenous molecules termed damage-associated molecular patterns (DAMPs) from dying cells. DAMPs trigger a cascade of inflammatory events that contribute to the activation of resident cells (microglia and astrocytes) and recruitment of circulating leukocytes (neutrophils, macrophages, dendritic cells, and T lymphocytes). Production of inflammatory mediators exacerbates neuronal injury. Conversely, activated resident cells might produce trophic factors that promote tissue repair and recovery. A potential role of regulatory T cells in restricting brain ischemic injury has been proposed [Liesz A et al. 2009].

Many studies suggest that inflammation has a deleterious effect [Fessbender K 1994, Hugs PM 2002] that amplifies ischemic injury and hence is a potential target for neuroprotective treatments to prevent cell death in the penumbra. However, some evidence suggests that inflammation is also involved in recovery and repair after ischemic brain damage, [Theod P 2009, Dennes A 2007] although this notion is controversial and needs further study.

Overall, a better understanding of the specific roles of inflammatory cells is needed, in particular about the kinetics of their recruitment and their relative contributions to each step of a stroke. Notably, the emerging and the intriguing role of different T-cell subsets needs to be established. Such information would help to define the therapeutic window in which the potential beneficial effects of inflammation in brain recovery in advanced stages of ischemia could be preserved (figure 1.2).

CNS and the immune system after a stroke Nervous and immune systems engage in a bidirectional communication that aims to maintain homeostasis in the whole organism. A stroke (as any acute lesion of the CNS) can disturb this generally well balanced interaction. Briefly, ischemic brain tissue releases factors such as cytokines and neurotransmitters that can reach chemosensitive brain areas involved in immune control such as the hypothalamus, [Meisel C 2005, Chomorro A and Dirnagl U 2007, Becker KJ and Emsley HC 2010] where they can in turn activate, for instance, the sympathetic nervous system. Damage to cortical regions involved in immune regulation, such as the insula, can lead to loss of tonic inhibition and thus activation of hypothalamic areas. Furthermore, inflammatory mediators can be released from the damaged brain tissue and enter the systemic circulation where they act on cells of the immune system in the blood and secondary lymphatic organs or, through the bloodstream, activate the brain via consensus. Besides immunomodulatory signalling specific to a brain lesion, [Catania A 2009] strokes are a strong unspecific stressor (eg, sudden loss of relevant bodily functions, fear, or sense of emergency) which activate immunomodulatory systems such as the hypothalamic–pituitary axis and the sympathetic nervous system [Elenkov IJ 2006] Within a few hours after the onset of cerebral ischemia, brain–immune system interactions can result in a downregulation of systemic immunity termed stroke-induced immunodepression (SIDS). Almost all immune cells have numerous noradrenaline receptors, which can be activated by circulating epinephrine produced by the adrenal medulla or via the dense innervation by postganglionic sympathetic fibers of lymphoid organs. Noradrenaline stimulates interleukin-10 production by blood monocytes. Overall, noradrenaline decreases the number and the activity of immune cells through its pleiotropic effects. Glucocorticoids, which are produced as a result of stress induced stimulation of the hypothalamic–pituitary-axis, are also well known immunosuppressants. After a stroke, patients often have impairments in swallowing and are therefore at high risk of aspiration. They are also exposed to the challenging microbial environment of intensive care. Infections are the most prevalent and relevant complications after a stroke: pneumonia is the main cause of death in such patients [Henon H, 1979] and has a substantial negative effect on recovery and rehabilitation. Since its description on clinical strokes, [CzlonKowska A 1979] many studies have confirmed the existence of SIDS in experimental [Prass K 2003] and clinical [Vogelgesang A 2008] strokes and shown a strong correlation between immunodepression, sympathetic nervous system activation, and outcome [Chammorro A 2007]. For example, concentrations of metanephrine in the blood are as robust for prediction of clinical outcome as they are for stroke severity, [Klehmet J 2009] and markers of SIDS, such as decreased

expression of HLA-DR on monocytes, predict risk of infection [Harms H 2008]. Nonetheless, immunodepression after a stroke might also have an adaptive component. As the result of a stroke, the blood–brain barrier is disrupted; CNS-specific antigens are exposed to the immune system and may enter the systemic circulation. Downregulation of the immune system could help prevent aggressive responses. Although the general response to a stroke could be a decrease in the number of immune cells and subsequently of their function, further complexity ensues as some immune-cell subtypes could increase (e.g., regulatory T cells). Little is known about the consequences of these changes in circulating immune cells in the brain, but there are indications that they might be involved in brain protection and repair [Gee JM 2007]. Immune responses against antigens are determined by the microenvironment of the tissue in which they occur. Co-stimulatory molecules are necessary for the priming of immune responses. Such molecules are weakly expressed in the healthy brain, but become upregulated after brain damage such as a stroke. Furthermore, systemic infection, which often occurs in patients after a stroke, leads to upregulation of co-stimulatory and MHC class I and II molecules in the periphery and the brain, thus facilitating activation of T cells and B cells against endogenous brain antigens [Becker KJ 2009]. As a result of systemic inflammation, for example, during infection cytokines are produced outside and within the brain and mediate aspects of sickness behavior [Dantzer R 2008]. Infection after a stroke might thus lead to an exacerbated proinflammatory phenotype. Within hours, a stroke induces systemic immune changes that last for weeks [Prass K 2003] and can affect clinical outcomes. SIDS at least partly explains the high risk of infection in patients after a stroke, and might thus be indirectly responsible for the production of inflammatory and co-stimulatory mediators that in turn negatively affect the brain lesion. Whether these deleterious effects of brain–immune interactions after a stroke are offset, at least partly, by their beneficial effect on brain repair or the restricted development of CNS integration is unclear.

Targets for Stroke Prevention: Role of Inflammation and Immunity

Numerous clinical trials have investigated the effects of anticoagulants (eg, low-molecular-weight heparins or warfarin), anti hypertensive molecules, antiplatelet agents (eg, aspirin, clopidogrel, Dipyridamole, ticlopidine, trifluoromethoxythienopyridine, GP IIa/IIIb inhibitors, or lotrafiban), or lipid-lowering drugs (eg, Statins) on prevention of stroke occurrence or recurrence [Stroke Trials Registry 2011]. Several of these strategies are presently being used to target the immune system. The MOSES trial, [Schrader J 2005] which tested the efficacy of an angiotensin type 1-receptor antagonist (eprosartan) versus a calcium-channel blocker (nitrendipine) in 1352 patients, showed that eprosartan reduced the risk of recurrent acute strokes by about 25%. Similarly, PROGRESS88 reported a 28% relative risk reduction (95% CI 17–38%; $p < 0.0001$) in the primary endpoint of cardiovascular events in 6105 patients who were randomly assigned to perindopril or control. Multiple trials have shown that anti-hypertensive drugs targeting the renin-angiotensin system can reduce stroke incidence. Beyond pharmacological strategies targeting this system, there are other interesting attempts to manipulate the immune system targeting molecules involved in blood-pressure regulation. PMD311789 and Cyt006-AngQb90 vaccines directed against angiotensin I and angiotensin II, respectively, have been tested in phase 2 trials and were inferior in their effect on blood pressure compared with pharmacological inhibitors of the renin-angiotensin system.

Nevertheless, strategies to improve the efficacy of such vaccines might confer substantial benefits for stroke prevention in the future by increasing the proportion of patients who are treated for hypertension, as these patients might more readily accept vaccination twice a year than pills every day. However, the long-term effects of such strategies remain unassessed [Brown MJ 2009]

Finally, because infectious agents seem to be risk factors for strokes, the argument for treating or preventing infection is well founded. For example, in one study [Hung IF 2007] dual pneumococcal and influenza vaccination was associated with a reduced number of cases of ischemic strokes than was reported in unvaccinated patients. Age dependent effects of vaccination, and the need to test, vaccination together with antiviral strategies such as use of neuraminidase inhibitors, are being discussed. Notably, prior infection has been suggested to increase hemorrhagic complications after thrombolysis with alteplase, but more data are needed [Urbanek C 2010].

Immunomodulation to Restrict Brain-Tissue Damage after a Stroke

There are strong experimental evidence which supports the role of leucocyte recruitment by adhesion molecules, cytokines, and chemokines in ischemic damage. Clinical studies support the therapeutic relevance of this pathway, and show increased ICAM1 expression in the cortex and increased circulating concentrations of soluble ICAM1 after an ischemic stroke [Rallidis LS 2009] In experimental models, co-infusion of antibodies targeting ICAM1 led to a significant improvement in neurological outcome when combined with tPA-induced thrombolysis, suggesting that ICAM1 blockade might improve functional outcome and extend the therapeutic window for thrombolysis [Bowes MP 1995]. Strategies have been designed to block the cytokine cascade that is induced after a stroke by use of recombinant human interleukin-1 receptor antagonist (interleukin-1Ra). Another promising strategy is the induction of mucosal tolerance with repeated low-dose intranasal administration of E-selectin, which causes a shift in immune responses from proinflammatory to anti-inflammatory and might even induce regulatory T cells. In rats, this immuno modulatory approach prevented ischemic and hemorrhagic strokes. The safety and effectiveness and maximal tolerable dose of E-selectin is under investigation in clinical trials [Emsley HC 2005, Takeda H 2002]

Other therapeutic approaches, such as the use of the antibiotic minocycline, showed promising results in animal models by reducing brain damage and promoting CNS repair [Tang XN 2007]. This tetracycline might exert anti-inflammatory activities through modulation of microglial activation, immune-cell activation, and thus the subsequent release of cytokines, chemokines, lipid mediators of inflammation, and MMPs [Brundula V 2002, Stirling DP 2005]. Minocycline might also reduce the harmful pro-bleeding effects of alteplase [Murata Y 2008]. Experimental evidence strongly suggests that both exogenous TPA (alteplase, injected intravenously) and endogenous TPA (produced and released by injured neurons) interact with the NR1 subunit of N-methyl-D-aspartate (NMDA) receptors, leading to increased glutamatergic signalling and subsequent promotion of excitotoxic or ischemic neuronal death. Strategies of active and passive immunization to prevent this interaction were shown to efficiently counteract the aggravating effect of TPA on excitotoxic or ischemic injuries [Macrez R 2010]. Although the translation from bench to bedside has not

been successful yet, strategies combining either anti-inflammatory drugs or neuroprotectants with thrombolytics are promising and need to be further studied.

Difficulties in Translation from Bench to Bedside and Research Priorities

With a few exceptions, positive laboratory results have not resulted in effective treatments for patients after a stroke. However, only a few trials have targeted inflammation or the immune system. What can we learn from past failures, and what are the prerequisites and prospects for future success in targeting inflammation and the immune system to prevent strokes, to minimize damage once it has occurred, and to foster regeneration and repair? First, the general question of whether the animal models of a stroke can recapitulate human pathology and predict success in clinical trials needs to be addressed. Many articles have assessed this point, [Dirnagl U 2006] which includes differences in vascular supply, kinetics of lesion evolution, confounding effects of anesthesia, difficulties in the assessment of functional outcomes, and deficiencies in the quality of preclinical trials. Accordingly, the Stroke Therapy Academic Industry Roundtable (STAIR) has provided substantial recommendations to improve the success of translation from bench to bedside. Some of these recommendations concentrate on the crucial differences between rodents and human beings and thus emphasized the need for studies into pharmacokinetics and pharmacodynamics, time to treatment, as well as inclusion of risk factors (especially ageing) and the need to use large animals [Fisher M 2005] Nonetheless, investigators need to consider specific issues about the translational roadblock when targeting immunological mechanisms. Animals used in stroke research live in conditions that are sterile or almost free of pathogens, and thus have comparatively little exposure to foreign antigens.

Moreover, rodents have a lymphatic differential blood count compared with human beings. Nevertheless, when direct comparison has been possible, it is reassuring that rodents had similar changes in adaptive and innate immunity to human beings after a stroke. However, to further improve prediction we should consider inclusion into our models of confounders related to inflammation, such as animals with atherosclerosis or pre-existing acute or chronic infections. A relevant concern relates to the potential risks involved with the use of therapeutic immunomodulation to treat strokes. Negative findings from other indications include life-threatening side-effects in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412 that was intended for the treatment of multiple sclerosis a rare but severe side-effect (progressive multifocal leucoencephalopathy) of the monoclonal antibody directed against the $\alpha 4$ subunit of integrins (natalizumab, an approved multiplesclerosis treatment), and meningoencephalitis as a side-effect of an amyloid- β vaccine in patients with Alzheimer's disease. Clearly, we need to be very alert to potential risks when targeting the immune system and carefully balance the risk-to-benefit ratios. Patients with chronic inflammatory diseases, such as rheumatoid arthritis, who might be at risk of infection or reactivation of viral infections, have successfully been treated with immunomodulatory therapies, such as rituximab, a chimeric anti-CD20 monoclonal antibody that depletes B cells. These findings prove that modulation of the immune system in elderly and severely ill patients, when done cautiously, can be safe. Another reason not to rush into clinical trials targeting the immune system in stroke patients lies in the fact that our current knowledge of the underlying pathophysiology is patchy at best. Importantly, although negative

consequences of brain inflammation after a stroke have been documented, there is some evidence that inflammatory mechanisms contribute to lesion containment or even regeneration and repair. The good and bad sides of inflammation after a stroke need to be clarified, and the differential kinetics of deleterious and potential protective or regenerative mechanisms need to be established. For example, MMPs, such as MMP-9, was reported to show deleterious effects during the stroke, but increased concentrations of these enzymes seem to promote brain recovery after a stroke. Accordingly, pharmacological approaches targeting MMPs (or any inflammatory mediator) need to be optimized for acute inhibition at the right moment (IE, to prevent damage without compromising beneficial effects). Furthermore, whether protection of one cell type necessarily protects other cell types in the neurovascular unit is not known. In this respect, it is encouraging that Dipyridamole, a platelet inhibitor used for stroke prevention, might also have anti-inflammatory and antioxidative effects, and might indirectly protect neurons through prevention of hypoxia-induced endothelial cytotoxicity. In terms of peripheral immunity in strokes, we are only beginning to understand the effects of the adaptive immune system in the pathophysiology of a stroke. Some effects, such as SIDS, which increases the risk for infection, are negative, but in other circumstances could balance, stroke-induced autoimmunity; for example, immune cells might react against brain epitopes that are normally well shielded but get exposed after a stroke. Novel investigations are needed to disentangle destruction and protection to safely and effectively modulate the immune system in patients after a stroke.

Experimental and clinical data justify cautious optimism that inflammation and immunity might one day be targeted to prevent strokes and to minimize its effects. In view of recent failures in the development of stroke treatments, the potential risks of such therapies, and the incomplete understanding of the underlying mechanisms, we advocate a cautious approach. Indeed, because of the complexities of the interactions between the immune system and the CNS during and after a stroke, it is difficult to pinpoint a mechanism as the most promising target or approach. Although immunomodulation of peripheral immune responses might carry a risk (e.g., the phase 1 clinical trial of TGN1412, which attempted to test anti-CD28 antibodies to boost regulatory T cells), we believe that such strategies could eventually lead to a viable approach to improve the stroke outcomes. Presently, clinical translation might be near for strategies that modulate innate immunity and inflammation in the brain, first through dampening deleterious hyperinflammation (classic anti-inflammation) or chronic inflammation (eg, pro-resolving drugs to overcome chronic inflammation and inflammation resolution deficits), and second by allowing inflammatory mechanisms to clear debris, restrict the spread of the lesion, and contribute signals that foster recovery and repair. Thus, a more comprehensive understanding of the endogenous immune responses, in particular with respect to different stages in the evolution of stroke pathology, from its prevention to recovery after a stroke, could help us to design safe immunomodulatory strategies with substantial benefits for patients.

Modulation of the Post-Ischemic Immune Response to Improve the Stroke Outcome

There are profound alterations in the systemic immune response following a stroke. The stroke induced changes include a dramatic increase in the systemic inflammation, as

measured by plasma concentrations of C-reactive protein (CRP) and interleukin (IL)-6 [Beamer NB 1995]. More recent data also demonstrates that a stroke leads to a sympathetically mediated dysfunction of the systemic immune response that predisposes to infection; the degree of these immunological alterations depends upon the stroke severity [Prass K 2003, Leisz A 2009]. Infection is common following a stroke, and the data demonstrate that infection in the post-stroke period, especially pneumonia (PNA), is an independent risk factor for poor outcome [Jhonston KC 1997, Aslayan S 2004, Katzan IL 2003]. Why infection predisposes towards a worse outcome is unclear, but our laboratory has focused on the possibility that an infection in this vulnerable time period may lead to changes in the microenvironment of the lymphoid organs and the brain that allow for the development of a detrimental immune response towards brain antigens. The immune response is generally thought of as having an innate, or antigen non-specific, an arm and an adaptive, or antigen specific, arm. The innate immune response is also referred to as the inflammatory response and is the first line of defense against invading pathogens and the primary response to tissue injury. It is thus not surprising that there is evidence of systemic inflammation following a stroke, and that the degree of inflammation correlates to stroke severity and ultimately to the amount of tissue infected [Adebert HJ 2004, Smith CJ 2004]. The amount of tissue infected is also reflected by the concentration of antigens like myelin basic protein (MBP), creatine kinase (CK)-BB, neuron specific enolase (NSE) and S100 in the peripheral circulation [Jauch EC 2006]. As concerns the systemic immune system, these antigens are essentially novel, which means that lymphocyte encounter with such and antigen could lead to the development of an autoimmune response. There are many potential sites of antigen encounter in the periphery, and experimental data show that antigen is present within the cervical lymph nodes shortly after the onset of a stroke [Vanzwam M 2009]. In addition, lymphocyte traffic into the infected brain tissue within days after a stroke, allowing for the possibility of novel antigen encounter within the central nervous system (CNS) itself.

For a lymphocyte to become activated to an antigen, it must generally be presented that antigen by a professional antigen presenting cell (APC) in the context of the major histocompatibility (MHC) II molecule and receive an additional costimulatory signal. The nature of the immune response is further shaped by the cytokine milieu of the environment at the site of antigen encounter. For instance, if a naïve lymphocyte recognizes its cognate antigen in the presence of interferon (IFN)- γ , a TH1 type response usually develops; if the recognition occurs in the presence of IL-4, a TH2 type response develops. Further, if antigen recognition occurs in the presence of transforming growth factor (TGF) - β 1 and IL-6, a TH17 response occurs; in the absence of IL-6, a regulatory T cell response (TREG) develops. A TREG response can also be induced in the presence of IL-10. As would be expected, the type of the effector cell/response generated after antigen encounter is associated with very different immunological outcomes. For instance, TH1 cells are associated with cellular immunity and are characterized by the secretion of IFN- γ and lymphotoxin alpha (LTA) while TH2 cells are associated with humoral immunity and the secretion of IL4, IL-5, and IL-13. And while TH17 cells play a role in host defense, they also appear to be important in the genesis of autoimmunity; TH17 cells are characterized by the secretion of IL-17 [Peck A 2008]. Inducible TREGs, on the other hand, are characterized by the secretion of TGF- β and IL-10 and, as the name implies, regulate immune responses.

To date, there has been little interest in exploring the possibility that autoimmune responses to brain might affect outcome from a stroke. There are, however, studies that

document the fact immune responses to brain antigens do occur following a stroke. For instance, lymphocytes from stroke survivors show more activity against myelin basic protein (MBP) than the lymphocytes from patients with multiple sclerosis [Younchayud U 1974]. In addition, myelin reactive T cells are found in higher numbers among patients with cerebrovascular disease [Wang WZ 1992]. These data thus provide evidence that a cellular immune response to brain antigens occurs following a stroke. Further, there are increased titers of antibodies to brain antigens, including neurofilaments and portions of N-methyl-D-aspartate receptor, following a stroke, indicating that there is also the development of a humoral response to these antigens [Bornestien NM 2001, Dambinova SA 2003]. The immune response to CNS antigens after stroke is likely just an epiphenomena of stroke, given that cerebral ischemic injury to the blood-brain barrier (BBB) allows for the systemic immune system to come into contact with the antigens that are normally sequestered from it. Nonetheless, it is possible that this response leads to “collateral damage”; whether these immune responses affect the outcomes of a stroke is a largely unanswered question. In an animal model of a stroke we have demonstrated that the predominant immune response to brain antigens, following a stroke is that of a TREG response characterized by secretion of TGF- β 1.23 Using the paradigm of mucosal tolerance, we also found it possible to induce TREG responses prior to stroke animals that exhibit a TREG response experience both better short (days) and long term (one month) outcome from a stroke [Gee JM 2008 and 2009, Zeerath D 2009]. It is thus not surprising that a recent paper found that the absence of TREG cells is associated with worse outcome from stroke [Liesz A 2009]. Induction of Treg cells characterized by the secretion of IL-10 prior to stroke also improves short term outcome and decreases infarct size. These data suggest that modulation of the post-ischemic immune response by inducing a more robust TREG response might be a viable strategy for neuroprotection. In our animal model, we also found that exposure to an inflammatory stimulus at the time of a stroke leads to a deviation of the immune response to that of a TH1 response, and the TH1 response to brain antigens is associated with worse outcome. This deviation from an endogenous TREG response to that of TH1 response induced by an inflammatory stimulus at the time of a stroke appears to be related to a change in the milieu of microenvironment at the site of antigen presentation. This observation may help to explain why persons who develop an infection in the post-stroke period have increased morbidity and mortality. It also suggests that attempts to avoid infection in the post-stroke period may be of therapeutic value. To date, however, there have only been a few trials of antibiotic prophylaxis following a stroke and these relatively small trials have conflicting results, suggesting that prophylactic antibiotic therapy may improve, worsen, or have no effect on the stroke outcome [Schwartz S 2008, Chomorro A 2005, Harms H 2008]. IL-17 was recently shown to contribute to ischemic brain injury up to 7 days after the onset of a stroke [Schichita T 2009] In this study, the IL-17 was being produced by $\gamma\delta$ T cells, which are important mediators in the antigen non-specific inflammatory response, and not by TH17 cells. Given the fact that there is robust upregulation of IL-6 following a stroke and increased expression of TGF- β 1 in brain, however, the post-ischemic cytokine milieu could favor the development of TH17 cells [Krupinski J 1996, Wang X 1995] Whether or not CNS specific TH17 cells contribute to injury in cerebral ischemia has yet to be explored, but their importance in autoimmune diseases, including experimental allergic encephalomyelitis (EAE), is clear [Furuzawa-Carballeda J 2007, Hoffstetter H 2009]. Recent data highlight the fact that the ratio of antigen specific TH17 cells to antigen specific TH1 cells as well as the cell type used

to induce EAE (TH17 versus TH1) influence both EAE phenotype and pathology [Jager A 2009, Stromnes IM 2008]. These data further suggest that the nature of the immune response to brain after a stroke has the potential to differentially affect outcome. The potential of modulating the post-ischemic immune response to improve outcome following a stroke is an intriguing neuroprotective approach as it is one of the rare interventions that could be initiated in a delayed fashion and still be expected to have an effect. There are, however, potential dangers of manipulating a response which is so complex and incompletely understood. As an example, we found that induction of MBP specific TREG cells prior to experimental strokes using the paradigm of mucosal tolerance was associated with better outcome up to one month after the ischemic injury; this benefit was not sustained to 3 months however, and there was a tendency for the animals “tolerized” to MBP exhibit a TH1 response to the antigen. Studies suggest that the phenotype of inducible TREG cells is unstable, so understanding how to prevent this “immunological drift” would be important prior to use in therapeutic trials [Zhu L 2009]. And while immunosuppressive strategies might decrease the risk of developing a TH1 (and possibly TH17?) response after a stroke, such interventions might increase the risk infection, a risk which is already high in the post-stroke period. On the other hand, strategies to enhance the immune response to prevent infection in the post-stroke period might increase the risk of developing a detrimental TH1 (and possibly TH17?) immune response to brain, and as already discussed, these responses might predispose patients to worse functional outcomes following a stroke. It is also in the realm of possibility that the development of immune responses to brain antigens, be they cellular or humoral, may have more long lasting effects. For instance, it is appreciated that a stroke is a potent risk factor for dementia, and it could be that autoimmune responses to brain contribute to cognitive decline and even the progression of white matter disease [Pendlebury ST 2009]. Future clinical studies will need to address the contribution of the post-ischemic immune response to these long-term outcomes. In summary, the nature of the post-ischemic immune response affects outcome from a stroke. Modulation of this response may be a viable approach to improving the outcome of a stroke, but there are potential dangers associated with immunomodulation. A more complete understanding of the endogenous immune response following a stroke is needed in order to safely manipulate this response in the post-stroke period.

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Chapter 12

PATHOPHYSIOLOGY OF STROKES

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ABSTRACT

Once considered exclusively a disorder of blood vessels, growing evidence has led to the realization that the biological processes underlying stroke are driven by the interaction of neurons, glia, vascular cells, and matrix components, which actively participate in mechanisms of tissue injury and repair. A stroke is a serious neurological disease, and constitutes a major cause of death and disability throughout the world. The pathophysiology of stroke is complex, and involves excitotoxicity mechanisms, inflammatory pathways, oxidative damage, ionic imbalances, apoptosis, angiogenesis and neuroprotection. The ultimate result of ischemic cascade initiated by acute stroke is neuronal death along with an irreversible loss of neuronal function. Therapeutic strategies for stroke have been developed with two main aims: restoration of cerebral flow and the minimization of the deleterious effects of ischemia on neurons. Intense research spanning over the last two decades has witnessed significant therapeutic advances in the form of carotid endarterectomy, thrombolytics, anticoagulant therapy, antiplatelet agents, neuroprotective agents, and treating associated risk factors such as hypertension and hyperlipidemia. However, the search for an effective neuroprotectant remains frustrating, and the current therapeutic protocols remain suboptimal. To date, only one FDA-approved drug is available for ischemic stroke; i.e., the serine protease tissue-type plasminogen activator (tPA), utility of which is limited by short therapeutic window. As new targets are identified, new opportunities emerge that build on an appreciation of acute cellular events acting in a broader context of ongoing destructive, protective, and reparative processes. The burden of disease is great, and its magnitude widens as a role

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for blood vessels and stroke in vascular and nonvascular dementias becomes more clearly established. The primary objectives of this chapter are: (1) to summarize the pathophysiology of a stroke, (2) to outline the relation of Atherosclerosis with stroke and also that of inflammatory and infective conditions associated with clinical stroke, along with role of various inflammatory mediators; (3) current status and understanding the role of genetics in stroke; (4) unifying the proposed mechanisms linking various pathogenetic processes; and (5) to discuss the emerging opportunities for novel therapeutic strategies. In this chapter we will critically evaluate the major mechanisms underlying stroke pathophysiology, with emphasis on potential novel targets for designing newer therapeutic modalities.

Keywords: Ischemic stroke; Pathophysiology; Excitotoxicity; Apoptosis; Inflammation; Therapeutic targets

INTRODUCTION

Strokes are the leading cause of disability worldwide, the second most common cause of dementia and the third leading cause of death. They have enormous clinical, social, and economic implications and demand a significant effort from both basic scientists and clinicians in the quest for understanding the underlying pathogenic mechanisms, and thereby adopting suitable preventive measures and successful therapies. A stroke is a sudden loss of brain function resulting from an interference with the blood supply to the central nervous system (CNS). Normal cerebral blood flow (CBF) is approximately 50–60 ml/100g/min. The reduction in CBF below 20 ml/100g/min results in an electrical silence and less than 10 ml/100g/min causes irreversible neuronal injury (Hakim M 1998, Jones TH 1981). Lack of blood circulation to the brain deprives neurons of necessary glucose and oxygen. Neurons are the impulse transmitters; hence they require a constant supply of energy. Up to 85% of all strokes are of ischemic origin, with most attributable to blockage of one or more cerebral artery by blood clots, with resulting reduction in cerebral perfusion. The remaining stroke cases are hemorrhagic, involving either intracerebral or subarachnoid hemorrhage. Ischemic stroke may manifest in the form of thrombotic stroke (large vessel and small vessel types); embolic stroke (with/without knowing cardiac and/or arterial factor); systemic hypoperfusion (Watershed or Border Zone stroke); or venous thrombosis. Irrespective of the cause, compromised vascular supply to the brain is the primary event in majority (85–90%) of acute strokes. A lower respiratory reserve and complete dependence on aerobic metabolism make brain tissue particularly vulnerable to the effects of ischemia. A spectrum of severity is generally observed in the affected region of the brain, owing to the presence of collateral circulation. Thus, part of the brain parenchyma (core) undergoes immediate death, while others may only be partially injured with potential to recover (penumbra). Following the onset of ischemic stroke, cerebral blood flow is disrupted throughout the affected region of the brain. Blood flow disruption to the tissue is not uniform due to the presence of collateral circulation resulting in a flow gradient. Consequently, a “core” region of infarct develops in which flow is severely reduced, often approximating upwards of a 90% decrement, and tissue undergoes necrosis within minutes as insufficient adenosine triphosphate (ATP) is present to maintain homeostatic ionic gradients and metabolic functions (Astrup J 1977). Surrounding this core region in which blood flow is severely reduced is the “penumbra”, a tissue

distribution in which flow is less severely reduced and remains typically around 35% of baseline flow (Astrup J 1977). Characterized by normal cellular membrane potential but a disruption in the ability for normal action potential firing, tissue initially residing within the penumbral region progresses to cellular death, ultimately resulting in an expanded core lesion without restoration of cerebral blood flow (Lo EH 2008). As such, therapeutic development has focused on achieving reperfusion via tissue plasminogen activator (tPA) and/or restoring homeostasis via administration of ‘neuroprotective’ pharmacologic agents intended to disrupt the ischemic injury cascade.

Few neurological conditions are as complex and devastating as stroke, the second leading cause of death worldwide. Also called a brain attack, victims may suddenly experience paralysis impaired speech, or loss of vision due to interruption of blood flow (ischemia) caused by thrombosis or embolism. Less frequently (<15%), strokes are caused by hemorrhage or cardiac arrest. On average, strokes in the USA strike once every 40 seconds and cause death every 4 minutes, with an estimated 41.6% death rate in 2007 (Lloyd-Jones, 2009). With an aging population, the absolute numbers are likely to rise. Among survivors, work capacity is compromised in 70% of victims, and 30% need assistance with self-care. Hence, the disease burden is great. The estimated stroke cost was 73.7 billion dollars in 2010 (USA) and projected to be 1.52 trillion dollars in 2050 (Lloyd-Jones, 2009). No racial or ethnic groups are spared, and the problem is global. For example, in the Russian Federation and China, the estimated death rates per 100,000 populations are five to ten times higher than in the USA (Lloyd-Jones, 2009). Hence, stroke is an affliction of mankind.

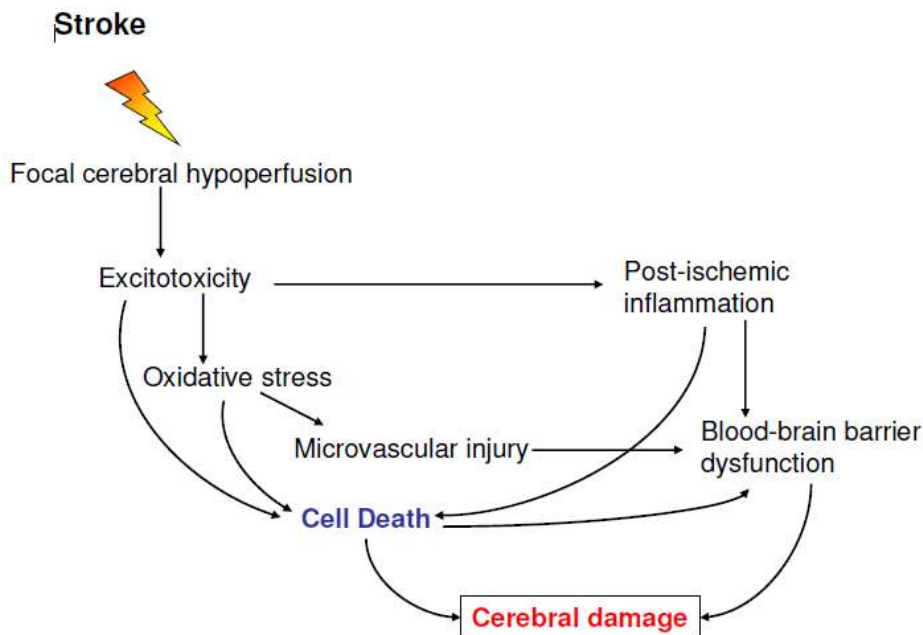


Figure 1. Ischemic cascade leading to cerebral damage: Ischemic stroke leads to hypo-perfusion of a brain area that initiates a complex series of events. Excitotoxicity, oxidative stress, microvascular injury, blood-brain barrier dysfunction and post-ischemic inflammation lead ultimately to cell death of neurons, glia and endothelial cells. The degree and duration of ischemia determine the extent of cerebral damage.

MECHANISMS OF NEURONAL INJURY

Cerebral ischemia results in a number of hemodynamic, biochemical, and neurophysiology alterations (Ostrowski RP 2006). A series of complex acute, subacute and chronic events occur after the incidence of stroke and reperfusion (Jean WC 1998). Ischemic injury involves energy failure, loss of cell ion homeostasis, acidosis, increased intracellular calcium excitotoxicity, free radical-mediated toxicity, and pathological permeability of the blood-brain barrier (BBB). Free radicals, specifically reactive oxygen species (ROS) that are generated soon after ischemia, as well as in later stages of ischemic reperfusion (e.g., by inflammatory cells), are the fundamental mediators of reperfusion injury.

The development of hypoxic-ischemic neuronal injury is significantly influenced by the release of excitatory neurotransmitters, primarily glutamate and aspartate in the brain. This process is called excitotoxicity and is activated by depletion of cellular energy stores. Glutamate, normally stored inside the synaptic terminals, is released into the extracellular space in a depleted energy state, which then results in the opening of calcium channels associated with N-methyl-D aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxanole propionate (AMPA) receptors. This leads to an influx of calcium, sodium and chloride ions and efflux of potassium ions. (Siesjo BK 1989, Hademenos GJ 1997, Garcia JH 1994). The influx of calcium is responsible for the activation of a series of destructive enzymes such as proteases, endonucleases, and lipases that allow release of cytokines and other inflammatory mediators, resulting in the loss of cellular integrity (Siesjo BK 1981, Becker KJ 1998). Inflammatory mechanisms play an important role in tissue injury by inducing the rapid production of many different inflammatory mediators. Within 30 minutes after ischemia and reperfusion, leukocytes recruited to the ischemic area activate mediators of inflammation such as oxygen free radicals, cytokines, and nitric acid.

Ischemia causes brain damage by activating the ischemic cascade, which progresses to local depletion of oxygen or glucose, causing failure of production of high energy phosphate compounds, like adenine triphosphate (ATP). This adversely affects energy-dependent processes necessary for tissue cell survival, and sets off a series of interrelated events culminating in cellular injury and death. The extent of damage usually depends on duration, severity, and location of ischemia. Neuron, owing to its role in impulse transmission, requires a constant supply of glucose and oxygen, in order to maintain the ionic gradients across its membrane, and is most susceptible to hypoxia changes. The various mechanisms involved in tissue injury/neuroprotection are:

- Failure of mitochondria leads to depletion of cellular energy store: This causes further energy depletion and may trigger cell death due to apoptosis. Ischemia also causes loss of potassium and ATP, which are essential for energy exchange. It has been observed that energy failure do not precipitate immediate cell death, but 5–10 min of occlusion may lead to irreversible brain injury. Most of the ischemic strokes do not usually cause complete occlusion of blood supply, however even a partial occlusion for prolonged period may cause harmful effect due to deterioration of ion gradient and by-products (like lactic acid, hydrogen ions) of anaerobic metabolism (Karaszewski JM 2009).

- Loss of membrane ion pump function and its deleterious effects: Ischemia, leading to inadequate energy supply at the cellular level, leads to malfunction of ion gradient, which results in loss of potassium in exchange of sodium, chloride, and calcium ions. This is accompanied by an inflow of water, resulting in rapid swelling of neurons and glia (cytotoxic edema).
- **Release of excitatory neurotransmitters:**
 - *Glutamate:* Ischemic cascade stimulates release of excitatory neurotransmitters in the brain, viz. glutamate and aspartate. Glutamate is vital for neuronal plasticity; however an uncontrolled release in ischemic areas mediate excitotoxic synaptic transmission via activation of N-methyl-d-aspartate (NMDA), 2-amino-3-hydroxy-5-methyl-4-propionate (AMPA) or kainite receptors, which allows Na^+ and Ca^{2+} influx. This has disastrous effect on the neuronal membrane, due to: (i) utilization of already depleted ATP in maintaining calcium balance; and (ii) causing disordered activation of a wide range of enzyme systems (proteases, lipases, and nucleases). These enzymes and their metabolic products, such as oxygen free radicals, damage cell membranes, genetic material, and structural proteins in the neurons, ultimately leads to cell death (Nakanishi N 2009). Understanding of these steps has triggered a search for potential blocking agents (Brouns R 2009). Release of excitatory neurotransmitter, glutamate, to modulate activity in nearby neurons occur through six known mechanisms: (i) reversal of uptake by plasma membrane glutamate transporters (Szatkowski M 1990), (ii) anion channel opening induced by cell swelling (Kimelberg HK 1990), (iii) Ca^{2+} -dependent exocytosis (Parpura V 1994), (iv) glutamate exchange via the cystine–glutamate antiporter (Warr O 1999), (v) release through ionotropic purinergic receptors (Duan S 2003), and (vi) functional unpaired connexons, ‘hemichannels’, on the cell surface (Ye ZC 2003). Though these various mechanisms of glutamate release have been elucidated, ambiguity still persists in terms of which of these mechanisms operate during normal physiological settings and which come into play during pathological settings like ischemia or stroke, since the latter may require conditions like cell swelling or a low extracellular Ca^{2+} concentration (Malarkey EB 2008).
 - *Synaptosomal-associated protein 25 (SNAP-25):* It is a neuron-specific protein, primarily localized in nerve endings and axons and involved in synaptic vesicle exocytosis, axonal outgrowth and transmitter release. Studies have shown that SNAP-25 is differentially regulated in ischemic stroke. Marti et al. (Marti E 1998) has demonstrated an increase in SNAP-25 mRNA levels in the infarct and penumbra of stroke patients, mainly during the first 6 days after stroke. The Ca^{2+} -dependent controlled exocytosis mechanism of glutamate release by the astrocytes is regulated by a complex of proteins, the soluble N-ethyl maleimide-sensitive fusion protein attachment protein receptor (SNARE) complex (Montana V 2004), which includes synaptobrevin 2, Syntaxin 1 and synaptosome-associated protein of 23 kDa (SNAP-23) (Parpura V 1995, Crippa D 2006) along with a Ca^{2+} sensor Synaptotagmin 4. Cultured astrocytes have been shown to exhibit expression of synaptobrevin II, cellubrevin and syntaxin but not SNAP-25 (Parpura V 1995). Astrocytes have also been observed to

contain certain proteins which are important for sequestration of glutamate into vesicles. These proteins include: vacuolar type H⁺-ATPase (V-ATPase), which is responsible for creating the proton concentration gradient necessary for glutamate transport into vesicles (Araque A 2000, Bezzi P 2001) and the three known isoforms of vesicular glutamate transporters (VGLUT): 1, 2 and 3, which use the proton gradient created by V-ATPases to package glutamate into vesicles (Freneue RT Jr 2002, Anlauf E 2005). The glutamergic exocytosis of astrocytes has been noted to be potential targets for various pharmacological agents and molecular biological manipulations (Montana V 2004).

- **Production of oxygen free radicals and other reactive oxygen species:** These react with and damage a number of cellular and extracellular elements, of which vascular endothelium is particularly important. It may also act by means of redox signalling to initiate the apoptotic pathway.
- **Apoptosis:** In contrast to necrosis causing cell death in the ischemic core, apoptosis (programmed cell death) occurs in the peripheral neurons. Ischemic damage causes an early response in gene expression of Bcl-2 and p53, followed by release of proapoptotic molecules such as cytochrome c and apoptosis-inducing factor from mitochondria. This leads to the activation of caspases and other genes that augment cell death (Joza N 2001, Ekshyan O 2004). The caspase cascade can be activated either by an extrinsic or death receptor-dependent route; and an intrinsic or death receptor-independent (mitochondrial) pathway (Eldadah BA 2000). Ischemia also activates various other signalling pathways which ultimately culminate in cell death. Prominent among these, include p53 (Mihara M 2003), JNK (of MAPK family (Gupta S 1995)), c-jun (Hayashi T 2000), p38 (Barone FC 2000) and cyclin dependant kinase 5 (cdk-5) (Green SL 1997), which are potential targets for therapeutic intervention.
- **Neuroprotection:** Ischemic cascade also activated various neuroprotective mechanisms as a defense against apoptotic and necrotic cell death. These include:
 - *Bcl-2* gene family: Includes antiapoptotic and proapoptotic counter molecules. Antiapoptotic members suppress release of sequestered proteins; and can also modulate calcium fluxes and caspase activation in the ER and inhibit the active form of Bax in mitochondria (Thominius MJ 2003).
 - Heat shock protein 70 (HSP70): HSP 70 is one of the earliest to be released. Its mRNA is expressed within 1–2 h of ischemia, with subsequent deregulation in 1–2 days. In animal models, HSP70 inducer, geranylgeranylacetone, has shown efficacy in limiting infarct volume. It also acts by increasing *Bcl-2* expression and inhibiting monocyte/macrophage activation (Gifard RG 2004, Yasuda H 2005).
 - Prion protein (PrPc): PrP mRNA is upregulated during hypoxia, and it inhibits Bax-induced cell death in neurons. Studies have observed that PrPc deletion results in reduced post-ischemic Akt phosphorylation, enhanced caspase-3 activation, with resultant exacerbation of ischemic neuronal injury in animals (Weise J 2006).

- Neurotrophin-3 (NT-3): Neurotrophins are growth factors, especially essential for the survival and maintenance of neurons, where NT-3 expression may be important in defining neuronal survival after brain ischemia. Animal studies have suggested that altered NT-3 expression following ischemia may be part of a physiological neuroprotective response after excitotoxic or ischemic damage (Yang JT 1998). Interestingly, continuous low-dose treatment with NT-3 protected against striatal neuronal loss in mild neonatal hypoxic/ischemic brain injury (Galvin KA 2003), suggesting that it might possibly be of therapeutic benefit in ischemic brain injury in man.
- Granulocyte-colony stimulating factor (G-CSF): The initial studies by Schabitz et al. (Shabitz WR 2003) have unraveled the neuroprotective potential of the potent haematopoietic factor G-CSF in an acute stroke model. Further studies have demonstrated the role of G-CSF in activating multiple cell survival pathways, reduction of infarct volume after stroke and possible role in improving long term behavioral outcome and plasticity after cerebral ischemia in animal models (Schenieder A 2005), while it has also been noted to offer protection to human cerebral neurons following *in vitro* ischemia (Jung KH 2006).
- Interleukin-10: IL-10 gene expression is elevated in association with most major diseases in the CNS and aids survival of neurons and glial cells by blocking the effects of proinflammatory cytokines and promoting expression of cell survival signals (Strle K 2001). Studies have also been suggested that following a cerebral ischemic attack, IL-10 may have a neuroprotective role (Spera PA 1998). It has also been reported that there is a significant reduction in serum IL-10 levels of stroke patients soon after an acute event, indicating that the anti-inflammatory response is subsequently downregulated in this subset of patients (Perini F 2001).

Knowledge of these mechanisms is vital in order to salvage brain tissue undergoing ischemic damage. Neuroprotective drugs that scavenge reactive oxygen species, inhibit excitotoxic neurotransmitters or inhibit apoptosis, if used during the ongoing phase of ischemic injury may help act to achieve this goal. Deep barbiturate coma, and recently described, NXY-059, the disulfonyl derivative of the radical-scavenging spintrap phenylbutylnitron, is reported to be neuroprotective in stroke (Fernandez Gomez 2008).

Concept of Ischemic Penumbra

Cerebrovascular tissue undergoing ischemia has two layers: (a) outer layer of less severe ischemia (penumbra), supplied by collaterals, and contain cells which can be retrieved by timely therapeutic intervention; and (b) inner core of severe ischemia with blood flow below 10–25%, displaying necrosis of both neuronal as well as supporting glial elements. Following an ischemic injury, the center of the core is perfused at 10–12 ml/100 g/min or less, while the ischemic area around it (surrounded by the penumbra) is critically hypo-perfused at less than 18–20 ml/100 g/min and is at risk of dying within hours. In contrast, the penumbra is perfused at least likely at approximately 60 ml/100 g/min and is less likely to die (Heusman PU 2003). Neurons in the penumbra are mostly dysfunctional, but may recover if reperfused

in time. This forms the basis of current protocols which favor early pharmacological intervention for re-canalization of occluded vessel, since it will not only salvage neuronal and glial cells from the penumbra, but also glial cells from the central ischemic core zone, thereby markedly limiting the size of infarcted tissue (Turner R 2007). (See below figure 2 of Ischemic penumbra)

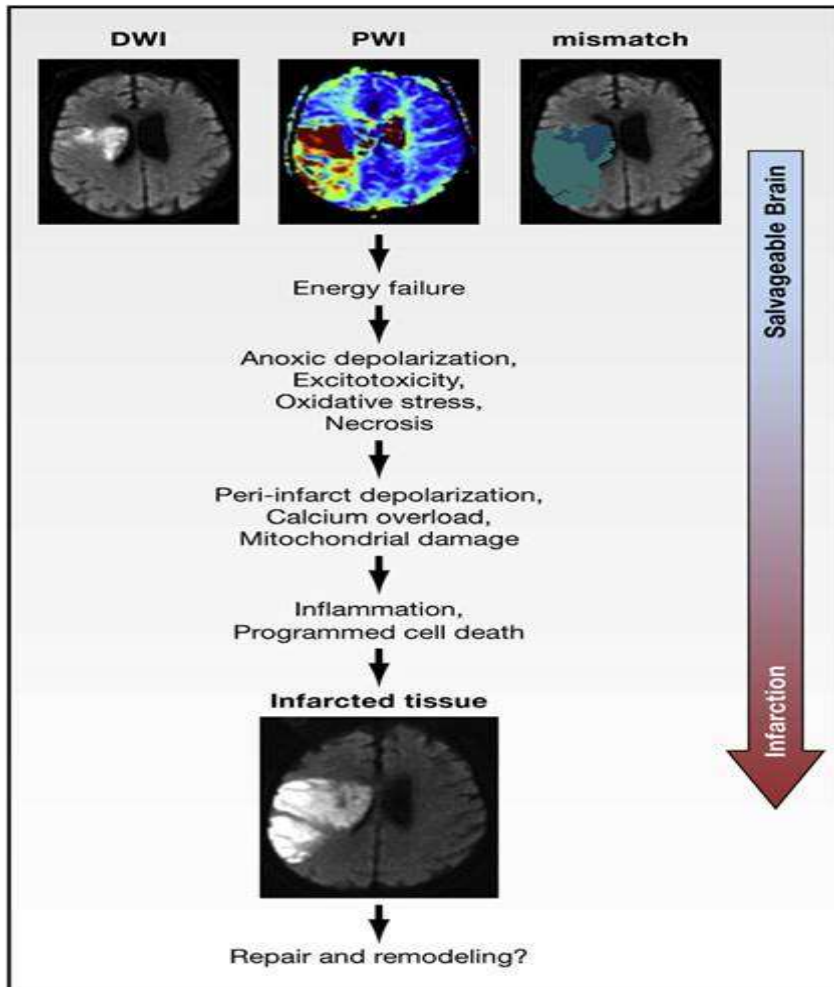


Figure 2. The Ischemic Penumbra: The ischemic penumbra represents compromised, but viable brain tissue lying distal to an occluded blood vessel. The top three MR images in this figure were taken in the same patient early after occlusion of the middle cerebral artery. The images are shown in the horizontal plane passing through the lateral ventricles. “Stunned, but not dead tissue” is detected by magnetic resonance imaging (MRI) techniques that assess (1) the spatial extent of the perfusion or blood flow deficit that presents as red and yellow areas on perfusion-weighted imaging (PWI, top middle image) and (2) severely damaged tissue in the ischemic core that presents as high-intensity areas on diffusion-weighted imaging (DWI, top left image) (Ebinger et al., 2009). In this patient, the perfusion deficit is larger than diffusion lesion. The top right image depicts the mismatch between core areas with a diffusion lesion (dark blue) and the larger areas with low perfusion (light blue-green). Over hours to days (see text), the core territory expands due to the complex cascades involving excitotoxicity, oxidative stress, programmed cell death mechanisms, and inflammation, as the initial trigger of energy failure ultimately progresses to infarcted tissue. The resulting infarct is shown in the image at the

bottom of the figure and is about the size of the initial perfusion deficit in this untreated patient. In later stages, some patients may also partially recover, in part due to endogenous mechanisms of repair and remodeling (Chopp et al., 2007). MRI scans courtesy of G. Alberts, Stanford.

CEREBRAL EDEMA AND ITS EFFECTS

This is of primary concern since it accounts for much of the death and disability. An understanding of the underlying mechanism, is important since management over the years have primarily addressed the effect without targeting the cause, which probably is attributable to the effects of neurogenic inflammation, mediated via neuropeptides, such as substance P. Klatzo classified edema as:

- Cytotoxic/cellular edema (Klatzo I 1987, Fishman RA 1992): Evolves within minutes to hours and are potentially reversible. It is characterized by swelling of all the cellular elements of the brain, including neurons, glia, and endothelial cells, due to failure of ATP-dependent ion (sodium and calcium) transport, as well as release of oxygen-derived free radicals.
- Vasogenic edema (Klatzo I 1987, Fishman RA 1992): Occurs over hours and days and are irreversible. It causes increased permeability of brain capillary endothelial cells to macromolecular serum proteins (e.g., albumin), resulting in an increase in extracellular fluid volume along with increased intracranial pressure (ICP).

This may displace the brain hemisphere; or shift one compartment of the brain, thereby compressing neurons, nerve tracts, and cerebral arteries. A sustained increase in pressure causes persistent ischemia; irreversible damage to brain cells; and, when severe may lead to cerebral herniation and potentially death. Initially acute hypoxia causes cytotoxic edema, which gives way to vasogenic edema with progression of infarction. This probably suggests that time is needed for the defects in endothelial cell function and permeability to develop.

EFFECTS OF ISCHEMIA ON STRUCTURAL INTEGRITY OF BRAIN

- Apart from the deleterious effect on brain cells, hypoxia also causes loss of structural integrity of brain tissue and blood vessels, partly through the release of proteases like matrix metalloproteases (MMP). Loss of vascular structural integrity results in the breakdown of the protective blood–brain barrier, manifesting as cerebral edema, along with secondary progression of brain injury (Adibhatla RM 2008). Studies have shown alterations of vascular endothelial receptor expression in ischemic stroke. Enhanced expression of vascular endothelial receptors is possibly mediated by both protein kinase C (PKC) and mitogen activating protein kinase (MAPK) which are the basis of treatment with PKC and MAPK inhibitors to limit the infarct volume (Edyinson L 2009).

- Brain ischemia also tends to activate the immune mechanism, which in some cases may be the cause for the exacerbation of damage and clinical deterioration of the patient. Experimental studies have shown that inhibition of the inflammatory process has led to the control of the extent of injury, an aspect which has gained paramount importance in understanding stroke and management of cases thereof (Ross R 1999, Ten Essecu R 2008, Adibhatla RM 2008).
- *Angiogenesis*: Extent of angiogenesis within the penumbra of ischemic stroke correlated with patient's survival time. This is mediated by either: (a) hypoxia inhibiting degradation of hypoxia-inducible factor-1 (HIF-1), which stimulates VEGF, which is essential for angiogenesis; (b) angiogenic growth factor secreted by inflammation-associated infiltrates [leukocytes, macrophages, damaged blood platelets] (Krupinski J 1994).

Hemorrhagic Stroke

This form of stroke occurs due to a blood vessel rupture in the brain. Its harmful effects are a resultant of: (a) hypoxia due to disrupted vascular supply; (b) irritant effect of releasing blood on brain parenchyma and vasculature; and (c) raised ICP due to continued bleeding, which may further restrict cerebral blood flow. In this respect, hemorrhagic strokes are more dangerous than ischemic strokes. There are two types of hemorrhagic stroke: intra-cerebral hemorrhage (generally occurs in small arteries or arterioles and is commonly due to hypertension, trauma, bleeding disorders, amyloid angiopathy, illicit drug use like amphetamines or cocaine, and vascular malformations), and sub-arachnoid hemorrhage (due to rupture of aneurysms from the base of the brain and bleeding from vascular malformations near the pial surface). It constitutes only 10–15% of all strokes (Escudero D 2008).

Atherosclerosis and Stroke

Atherogenesis is a decade-long process which involves luminal obstruction by cellular and extracellular substances. The pathogenetic process from onset of atherosclerotic changes in cerebrovascular or extracranial circulation to precipitation of acute ischemic stroke with its consequent cell damage is complex and many of the intermediary steps are not fully understood. Changes may manifest in the form of: (a) *fatty streak*, earliest lesions seen as yellowish areas of discoloration of intima, due to accumulation of lipid-filled macrophages (foam cells) in approximately 30% children below 5 years (Stary HC 1987); (b) *more advanced lesions with massive extracellular lipid* at the branching points of arterial vessels, in late childhood and early adolescence (Ip JH 1990); (c) *complicated fibrous plaques*: central acellular area of lipid covered by a cap of smooth muscle cells and collagen, seen in the third decade of life (IP JH 1990). Persons with risk factors for atherosclerotic disease (e.g., hypertension, hypercholesterolemia, cigarette smoking) tend to have clinically advanced atherosclerotic lesions with increased frequency. Sequence of events in atherogenesis is:

1. Injury to arterial wall: In his “response-to-injury” theory, Ross had hypothesized that, atherosclerosis is the effect of the complex interplay among monocytes, lipoproteins, platelets, lymphocytes, and smooth muscle cells in the intimal layer (Ross R 1999). Ip et al. In 1990 put forth a three-tier pathophysiologic classification system:

- *Type I injury:* Chronic minimal injury with functional alterations of endothelial cells without significant morphological changes, primarily caused by the turbulence of blood flow. Other contributory factors being hypertension, hypercholesterolemia, circulating vasoactive amines, immunocomplexes, viral infections, and tobacco smoke.
- *Type II injury:* Endothelial denuding and superficial internal injury, possibly due to toxic products released by accumulating macrophages in the intima. These changes may be accompanied by platelet deposition with or without thrombus formation, and subsequent thrombus incorporation.
- *Type III injury:* Manifested by deep intimal and medial damage, accompanied by marked platelet aggregation and mural thrombosis, generally following plaque rupture.

2. Role of monocytes and T-lymphocytes in foam cell transformation:

The next important events are:

- *Circulating monocyte adhesion:* Abnormal shear stress at atherosclerotic lesion-prone sites in the circulatory system enhances the production of certain transcription factors, which promotes the expression of endothelial vascular cell adhesion molecule (VCAM), which is imperative for monocyte binding to endothelial cells (Gimbrone MA Jr 1995).
- *Monocytes insinuation* between the tight junctions of the endothelial cells to enter the subendothelial space.
- *Activation of immune mechanism:* In the form of T-lymphocyte activation, in both early fatty lesions and in advanced fibrous lesions, which helps monocytes migration and its transformation into “foam cell”.

3. Oxidation of LDL-cholesterol: Oxidation of LDLcholesterol induced by free radicals produced by macrophages, endothelial cells, or smooth muscle cells, participates by:

- a) Formation of foam cells;
- b) Cytotoxic properties promoting endothelial injury;
- c) Chemoattractant for circulating monocytes; and
- d) Inhibiting egress of macrophages from plaques (Adibhatla RM 2008).

4. Smooth muscle cell migration and proliferation: A number of molecular factors may play a role in this vital plaque-forming process. They include growth factors (e.g.,platelet-derived growth factor, or PDGF, a polypeptide released from blood platelets and endothelial cells that may attract smooth muscle cells to the intima and encourage them to divide); eicosanoids (which can stimulate the hydrolysis of

cholesterol ester, producing free cholesterol); certain cytokines (e.g., tumor necrosis factor, interleukin-1 and interferon); and nitric oxide which acts to dilate blood vessels.

5. **Role of platelet:** Platelet aggregation and adhesion promoted by toxic products released by macrophages and by moderate damage to the internal surface with denudation of the epithelium have vital roles in the progression of atherosclerosis (Ip JH 1990).
6. **Plaque fissuring and thrombus formation:** The process of plaque destabilization (fissuring and rupture, followed by thrombus formation) is not fully understood.
 - **Plaque fissuring is possibly due to:**
 - a) Loss of an internal lattice of collagen supporting the cap of the plaque., making it vulnerable to circumferential stress during systole;
 - b) Infiltration of the cap tissue with foam cells, which possibly weakens the tissue by passively distorting the spatial arrangement of the connective tissue matrix or by actively destroying connective tissue matrix protein by lyric mechanisms (Slager CJ 2005).
 - **Thrombus formation occurs by:**
 - **Platelet activation:** On coming in contact with the sub-endothelial collagen, exposed by way of plaque rupture, circulating platelets get activated, and subsequently aggregate and adhere, through interaction with platelet surface receptors (most important being GP-Ib-IX) and sub-endothelial protein ligand von Willebrand factor (VWF). An association between elevated vWF concentrations and arterial thrombosis has been described (Jansson JH 1991).
 - **Platelet activation and blood flow:** Under high fluid shear-stress conditions, as in atherosclerosis, there is evidence that platelet aggregation depends on the binding of VWF to platelet GPIIb/IIIa, blockade, of which inhibits platelet aggregation and thrombus formation without disturbing the initial platelet adhesion (Stoll G 2008).
 - **Activation of the coagulation cascade:** Endothelial injury constitutes the primary impetus for the initiation of the extrinsic pathway of the coagulation cascade. Activated platelets, apart from forming the backdrop of the clotting process, also acquire enhanced capacity to catalyze interactions between activated coagulation factors (which normally circulate as inactive precursors: *zymogens*). Factor Xa, is the active catalytic component of the “prothrombinase” complex, which converts prothrombin to thrombin. Thrombin cleaves fibrinopeptides (FPA, FPB) from fibrinogen, allowing the resultant fibrin monomers to polymerize, and converts Factor XIII to XIIIa, which crosslinks (XL) the fibrin clot. Thrombin accelerates the process by its potential to activate Factors V and VIII, while a number of natural plasma inhibitors retard clotting, including C1-inhibitor (C1 INH), tissue factor pathway inhibitor (TFPI), and antithrombin II (ATIII). The fibrin molecules aggregate together, trapping platelets, erythrocytes, and leukocytes to form the thrombus. Subsequently, there is a tendency for the clot to enlarge as blood flow slows around it, resulting in enlargement of size (“*propagating thrombus*”).

- **Physiologic-subtypes of thrombosis related ischemic stroke:**

Ischemic stroke with different etiologies, possibly have a link based on the process of thrombosis:

- Atherothrombotic occlusion of larger arteries (e.g., carotid, middle cerebral, basilar): Most common causes of primary large vessel occlusive cerebrovascular disease, and also is the most common cause of stroke.
- Embolism is the second most common cause of stroke. Most are due to cerebral arterial atherothrombosis; or may also arise from other cardiogenic sources and deep vein thrombosis.
- Microatheroma of an influx of fat-like materials (lipohyalinosis) affects small vessels; and most prominently causing lacunar strokes.

7. *The potential outcomes of plaque fissuring:* It includes:

- a. Sealing of the fissure with fibrosis of incorporating thrombus; or
- b. Mural intra-intimal and intra-luminal thrombosis, resulting in partial or transient reduction in blood flow (precipitating transient ischemic attacks: TIA). It may progress to occlusive thrombosis, which, if persistent due to absence of collateral flow, can lead to ischemic stroke (IP JH 1990).

8. *Evolution of cerebral atherothrombosis:* Thrombosis may evolve over a few minutes, or take hours or even days. A stroke that is actively progressing as a direct result of increasing occlusion and ischemia is termed '*stroke in evolution*' or '*progressing stroke*'. A large blood vessel may evolve over a longer period of time, as compared to a smaller vessel, and there may be warning signs like TIA. Damage in ischemic stroke may result not only from infection, but also from edema, which generally peaks in 2–5 days. (I) *Hemorrhagic conversion*: Natural consequence of reperfusion of blood through damaged blood–brain barrier, in ischemic stroke, generally has 'bland' infarction associated with secondary bleeding. This is referred to as hemorrhagic conversion or hemorrhagic transformation (HT), and is characterized by gross parenchymal hematoma with possible intraventricular extension, midline shift and herniation; along with widespread leukocyte infiltration and macrophage invasion (Teal PA 1992). HT may be seen as hemorrhagic infarction (HI) or, less commonly, parenchymatous infarction (PH). Though both have different incidences, pathogenesis, and clinical outcome, but distinguishing HI and PH on CT may be difficult. On CT, HI appears as a discontinuous heterogeneous mixture of high and low densities occurring within the vascular territory of the infarct. In contrast, PH appears as a discrete, homogeneous collection of blood that often exerts mass effect and may extend beyond the original infarct boundaries or even into the ventricles (Teal PA 1992).

Recent studies have suggested the role of an abnormal expression of some matrix metalloproteinases (MMPs) which can degrade almost all components of the extracellular matrix and basal lamina such as laminins, fibronectin, or type IV collagen, weakening brain microvessels and predisposing them to rupture and increasing the risk of cerebral hemorrhage and precipitate hemorrhagic transformation events after stroke (Gasche Y 1992). Previous animal models (Heo JH 1999, Rosenberg GA 1996) have demonstrated an abnormal

expression of MMP-2 (Galatians A) or MMP-9 (Galatians B) after cerebral ischemia and in lipopolysaccharide-injured brains (Mun-Bryce S 1998), contributing to brain injury and BBB breakdown. Further, Asahi et al. 2001 and Sumii et al. 2002 reported that pharmacological or genetic inhibition of MMP-9 has caused a significant reduction in size of the infarct, as well as the risk of hemorrhagic complications, while Wang et al. 2003 and Tsuji et al. 2005 noted tissue plasminogen activator-associated hemorrhage and edema appeared to be correlated to MMP-9 dysregulation. Investigations dealing with ischemic stroke in human subset have revealed high MMP-9 levels in peripheral blood, with MMP-9 levels showing correlation with poor neurological outcome, infarct growth, and hemorrhagic transformation events (Montaner A 2001, 2003). Rossel et al. 2008 have reported a strong neutrophil infiltration in the infarcted and hemorrhagic areas with local high MMP-9 content closely related to basal lamina collagen IV degradation and blood–brain barrier breakdown. Hemorrhagic infarction (HI) occurs regularly in the natural evolution of acute embolic stroke and is usually asymptomatic. Autopsy studies of HI may vary from patchy petechial bleeding to more confluent hemorrhages, with an occurrence rate ranging from 51% to 71% of recent embolic strokes (Teal PA 1992). According to another estimate (Leonard AD 1992), approximately 20% of patients with cardioembolic stroke have hemorrhagic transformation in the infarcted zone, usually occurring within 48 h, and is rare in the first 6 h. HI has been often explained as a result of reperfusion of the vascular bed of the infarct, such as would occur after fragmentation and distal migration of an embolus or after early reopening of a large vessel occlusion in the setting of a large infarction; the full pressure of arterial blood into hypoxic capillaries results in a diapedesis or red cells through their hypoxic walls (Mohr JP 1992). It has been observed that HI may develop in areas distal to a persisting occlusion, suggesting that reperfusion is not always a prerequisite for this phenomenon. Many studies have found strong correlation with risk factors like:

- 1) One or more surges of arterial hypertension;
- 2) Associated hyperglycemia; and
- 3) Restoration of blood flow to ischemic territories.

Possible mechanism for this is marked tissue energy depletion accompanied by acidosis damaging brain vessels, causing leakage of edema fluid and red blood cells.

Parenchymatous infarctions (PH) occur less frequently, but are often symptomatic due to extension and mass effect beyond the original infarct territory (Teal PA 1992). In contrast to HI, it has been proposed that the pathogenesis of PH may involve “ischemic necrosis resulting in the rupture of small penetrating vessels analogous to hypertensive hemorrhage, leading to massive bleeding”. Trials of thrombolytic therapy for acute ischemic stroke, has revived interest in this, since PH appears to be associated with anticoagulation therapy (Adhi bhatla RM 2008, Pandian JD 2009).

Free Radicals in Cerebral Damage

A vast amount of data implicates oxygen-derived free radicals (especially superoxide and hydroxyl radicals) and high-energy oxidants (such as peroxynitrite) as mediators of inflammation in ischemia/reperfusion injury (Cuzzocrea S 2001). Free radicals are highly

reactive molecules generated predominantly during cellular respiration and normal metabolism. Imbalance between cellular production of free radicals and the ability of cells to defend against them is referred to as oxidative stress (Valko M 2007). After brain injury from ischemic stroke, the production of ROS dramatically increases, leading to tissue damage via several different cellular, molecular pathways, and mechanical pressure (Figure 3).

ROS can cause damage to cellular components such as lipids, protein, and nucleic acids, leading to subsequent cell death (Janardhan V 2004). Damage can become more widespread due to weakened cellular antioxidant defense systems in ischemia. ROS are the major stimulators of inflammatory cytokine production (such as interleukin-1, tumor necrosis factor- α , and interferon- γ) and protease secretion by microglia, leukocytes, and resident cells of the neurovascular unit (Haddad JJ 2002). As these neuroinflammatory mechanisms become activated, alterations in cytokine profiles, adhesion-molecule expression, and tight-junction components mediate further vascular leakage. Significant evidence exists suggesting that ROS are involved in every fundamental physiological step that leads to neuronal death (Cao W 1988) and thus are considered an important target in developing an effective stroke therapy (Juurlink BH 1997).

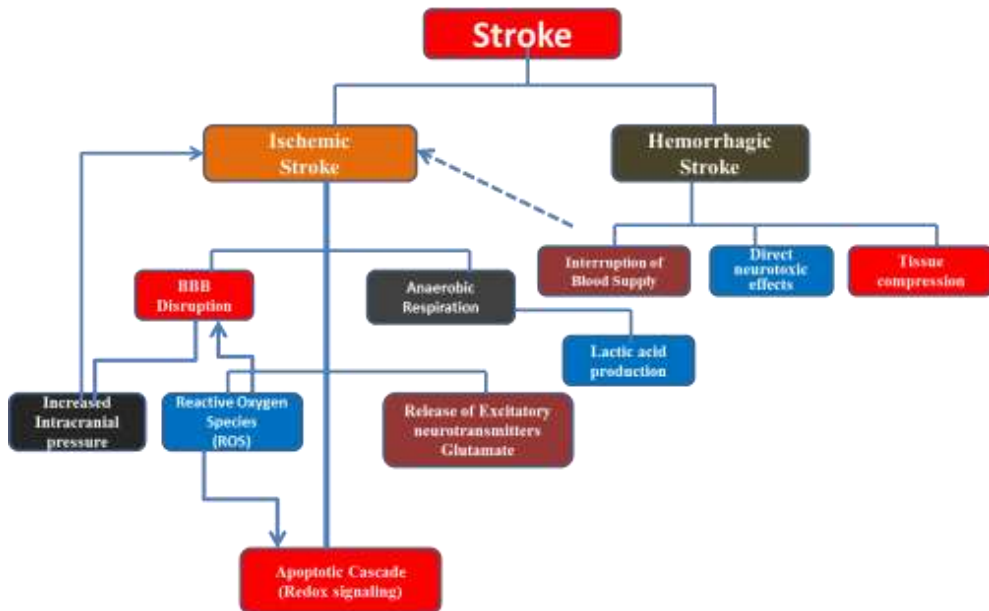


Figure 3. Schematic showing different types of stroke and cascade of events following ischemia.

Oxidative and Nitrosative Stress Oxidative and nitrosative stress are also powerful mediators of ischemic injury. In one scenario, the redox environment of cells modulates signal transduction cascades that tip the balance between prodeath and prosurvival pathways (Crack and Taylor, 2005). In the second scenario, ROS and perhaps reactive nitrogen species, including peroxynitrite, act directly as executioners of cell death (Chan, 2001). The brain appears particularly vulnerable to radical-mediated attack because of its limited antioxidant defenses (Adibhatla and Hatcher, 2010). Until recently, it was accepted that during ischemia ROS were generated principally by mitochondria, a well-known ROS source during electron transport and oxidative phosphorylation. However, it was recently shown that NADPH

oxidase generates the majority of superoxide anion produced *in vivo* and *in vitro* during NMDA receptor activation and ischemia (Brennan, 2009; Girouard, 2009). Thus, the mitochondrion does not appear to be the major source of radicals following NMDA receptor activation. However, the close proximity of NADPH oxidase to neuronal mitochondria (Girouard, 2009) raises the possibility that this enzyme generates “kindling” ROS that promote mitochondrial uncoupling, triggering a secondary ROS surge from mitochondria (Figure 3). These observations point to NADPH oxidase as a potentially important therapeutic target in stroke but also highlight the need for a better understanding of the interaction among the different ROS sources and the relative contribution of vascular versus neuronal sources. In this regard, a surprising finding has been that activation of NMDA receptors increases ROS both in neurons and vascular cells (Girouard, 2006), suggesting a role of vascular ROS in excitotoxic brain damage. Although this observation emphasizes the close relationships between vessels and neurons (see Protecting the Neurovascular Unit), it has not been established how vascular oxidative stress may contribute to tissue outcome in excitotoxic brain injury.

NO and related oxidation products are key players in excitotoxicity. Reactive nitrogen species have important cellular effects, such as inhibition of key mitochondrial enzymes, facilitating mitochondrial transition pore formation, DNA damage, PARP activation, and activation of Ca^{2+} -permeable TRPM7 channels (Aarts and Tymianski, 2005; Pacher, 2007). As a potential second mechanism, NO modifies protein groups by covalently attaching two cysteine residues forming an S-nitrosothiol derivatives. This posttranslational modification significantly impacts cell survival by altering, for example, the function of critical regulatory proteins such as campuses, metalloproteases (GU et al, 2002), and the glycolytic enzyme GAPDH (Nakamura and Lipton, 2009). As NO has the potential to target a number of specific cysteine residues to alter protein function, the impact of S-nitrosylation is (largely) underexplored in the ischemic process. Taken together, the experimental evidence implicating oxidative and nitrosative stress in stroke is strong. But the translation of these fundamental concepts into clinical applications has proven to be challenging. Most recently, a nitron-based radical spin trap failed in the final phase 3 clinical trial for acute ischemic stroke (Shuaib, 2007). And more broadly, the history of antioxidant therapies in clinical trials for neurodegeneration has been littered with many disappointments (Kamat, 2008). Overproduction of reactive nitrogen and oxygen species are surely damaging to brain cells. But at lower, homeostatic levels, radicals are critical signaling molecules participating in normal neuronal and vascular function (Faraci, 2006). Finding novel ways to scavenge or suppress deleterious radicals without interfering with endogenous signaling will be important to design effective therapies. As discussed above for excitotoxicity, a desirable strategy would be to develop drugs that only become activated by the pathological state intended for inhibition, i.e., during oxidative stress (Lipton, 2007b). Another approach would be to test antioxidant molecules with pleiotropic properties, such as activated protein-C (APC). APC blocks ROS generation, suppresses postischemic inflammation, and blocks apoptosis in neurons and endothelial, thereby providing both parenchymal and vascular protection (Yamaji, 2005; Cheng, 2006; Liu, 2004). APC as well as other pleiotropic agents with a broad spectrum of activity in experimental stroke are undergoing clinical trial testing (Ginsberg, 2009).

Excitotoxicity

Cerebral ischemia initiates a cascade of detrimental events including glutamate associated excitotoxicity, membrane lipid degradation, DNA damage, formation of reactive oxygen species and acute inflammation, which lead to the disruption of cellular homeostasis and structural damage of ischemic brain tissue. Excitotoxicity and calcium overload are major factors contributing to the early stages of ischemic cell death. The canonical pathway asserts that glutamate, the most abundant neurotransmitter, accumulates into the extracellular space as a consequence of energy and ion pump failure, as well as the failure of re-uptake mechanisms (Choi and Rothman, 1990). The glutamate overload leads to prolonged stimulation of AMPA and NMDA ionotropic receptor subtypes to dramatically enhance the influx of calcium, sodium, and water into neurons. Massive calcium influx activates catabolic processes mediated by proteases, lipases, and nucleases (Ankarcrona, 1995). In addition, activation of nNOS, PLA2, and other Ca^{2+} -dependent enzymes leads to production of NO, arachidonic acid metabolites, and superoxide, which act as additional triggers of cell death (Dirnagl, 1999; Lo et al, 2003). For these and other reasons, oxidative phosphorylation becomes uncoupled; leading to further ATP depletion, ROS production, and release of stored Ca^{2+} from mitochondria, further accelerating a series of catastrophic events that lead to acute cell death. Despite the validation of glutamate toxicity in the laboratory setting and its enormously positive impact on the quest for new therapeutics in neuroscience, numerous clinical trials targeting NMDA and AMPA receptors have failed to improve outcome in stroke patients (Ginsberg, 2009). Although replete with shortcomings in trial design and implementation, the failure to translate has reinforced the notion that the glutamate hypothesis may have oversimplified the complexity of the cell death process and underestimated the diversity of expressing cell types as well as the heterogeneity of glutamate responses following receptor overstimulation in stroke. Moreover, it is likely that NMDA-AMPA pathways comprise only a subset of routines disrupting ionic imbalance following glutamate receptor activation. As originally conceived, the NMDA-AMPA model did not consider the delayed and invariably fatal glutamate-independent calcium influx following oxygen-glucose deprivation or the failure of the glutamate receptor blockade to halt cell death after very intense oxygen-glucose deprivation (Aarts, 2003).

Equally importantly, it could not account in an obvious way for the death of non-neuronal cell types such as astrocytes, vascular cells, or microglia in ischemic tissue. An alternative viewpoint is that the drugs tested in clinical trials were poorly chosen, because many of them block all receptor functions (Lipton, 2007a). As a result, the dosing may have been suboptimal, due to the development of untoward side effects that prevented dose escalation to neuroprotective levels. A more suitable approach would be to use therapeutic agents that selectively target excessive channel opening (e.g., uncompetitive inhibitors with a relatively fast offrate, such as mountain (Orgogozo, 2002; Mobius and Stoffler, 2002; Lipton, 2006; Kavirajan and Schneider, 2007), thereby retaining normal receptor functions (Lipton, 2007a).

To add to the complexity, it is now known that NMDA receptor location and subunit composition differently regulate neuronal survival or death, although the strength of the calcium signal may specify the fate of neurons best following NMDA over-activation (Stanika, 2009). With notable exceptions, selective enhancement of synaptic receptors (in which NR2A peptide subunits predominate over NR2B) promotes neuronal survival, whereas activating extra synaptic receptors (in which NR2B peptide subunits predominate over

NR2A) promote cell death (Chen, 2007), in part, by activating death-associated protein kinase 1 (Tu, 2010). Turning CREB-dependent signaling and BDNF expression up or down is critical for cell fate determination during NMDA receptor activation. The MAP kinase family members P38 and ERK1/2 participate, most likely by phosphorylation-dependent regulation of sub-membrane scaffolding proteins and NR2A and NR2B receptor subunits (Hardingham, 2002). Under ischemic conditions, the vulnerability to cell death is reduced when postsynaptic scaffolding proteins are modified, and PSD-95 binding is prevented (Cui, 2007). Moreover, successfully targeting the glutamate receptor complex early on would also diminish the opening of the downstream channels, e.g., transient receptor potential channels (TRP) and acid-sensing ion channels, as well as suppress the frequency of cortical spreading depolarizations (see below), ongoing within the ischemic penumbra. Hence, understanding and leveraging the repertoire of diverse responses and mediators at the receptor and second messenger level can only enhance possibilities for successfully targeting excitotoxicity for the treatment of ischemic injury.

Calcium Dysregulation: It is also known that increased calcium influx from glutamate receptor overactivation combined with release of Ca^{2+} from mitochondria and other stores (e.g., endoplasmic reticulum) may not fully account for the irreversible buildup of intracellular Ca^{2+} after excitotoxic stimulation. Other channels and ion pumps activated during ischemia in addition to glutamate receptors have been implicated in the Ca^{2+} accumulation. These include the $\text{Na}^{+}/\text{Ca}^{2+}$ exchange (Bano, 2007) hemichannels (Contreras, 2004), acid-sensing ion channels (Xiong et al., 2004), volume-regulated anion channels (Kimelberg et al., 2006; Simard, 2007), and TRP channels (Aarts and Tymianski, 2005). In particular, ASIC1a, activated by ischemia-induced acidosis, is involved in the Ca^{2+} influx and its inhibition is neuroprotective (Simon, 2006). ASICs are stimulated within the pH range commonly found in ischemic brain tissue, thus explaining the well-established link between acidosis and worsening of ischemic outcome in animals and humans. Failure of Ca^{2+} efflux mechanisms, especially the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger also contributes to the Ca^{2+} accumulation. Prostaglandin E2 EP1 receptors have a role in the failure of the $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger during ischemia, and their inhibition is markedly neuroprotective (Abe, 2009; Kawano, 2006). Therefore, targeting Ca^{2+} influx-efflux mechanisms may be a valuable approach to counteract Ca^{2+} dysregulation and associated injury in conjunction with novel drugs targeting glutamate receptors.

Cortical Spreading Depolarizations

In addition to excitotoxic events at the molecular and cellular level, massive release of glutamate and ionic imbalance negatively impact the evolution of ischemic injury at the tissue level. Cortical spreading depolarization (CSD), a high energy consuming phenomenon akin to the cortical spreading depression of Leao, is triggered by high levels of extracellular glutamate and K^{+} and is characterized by slowly propagating massive depolarization of neurons and astrocytes along with drastic disruption of ionic gradients (Somjen, 2001). Electrophysiological and imaging data convincingly demonstrate CSD in patients with ischemic stroke (Dohmen, 2008). In experimental animals, a single cortical spreading depolarization expands the area of severe hypoperfusion by more than 20% (Shin, 2006). In fact, it appears that a greater frequency, but more importantly, duration of DC shifts

correspond to accelerated infarct maturation and core expansion (Mies, 1993; Back, 1996). The infarct expansion is probably due to mismatch between high metabolic demand to support membrane re-polarization and marginal tissue perfusion due to constrained penumbral blood flow (Back, 1996). Hence, suppressing CSD may prove worthwhile for reducing infarct maturation. Notably, the noncompetitive NMDA receptor antagonist ketamine markedly suppressed CSD frequency in head injury patients (Sakowitz, 2009), but it is unclear whether it improved clinical outcome. By contrast, irreversible depolarizations resistant to NMDA receptor antagonists (anoxic depolarization) developments in the ischemic core territory (Nellgiad and Wieloch, 1992) and are probably terminal events contributing to severe ionic imbalance and tissue failure.

INFLAMMATION

Focal ischemia evokes a robust inflammatory response that begins within a few hours of onset and typifies the secondary or delayed response to ischemia. It involves the activation of microglia and astrocytes as well as an influx of hematogenous cells recruited by cytokines, adhesion molecules, and chemokines across the activated blood vessel wall. Stimulated brain cells and blood vessels then communicate with one another through a complex network of paracrine and autocrine signaling. The highly choreographed response evolves over many days, with multiple signals and targets triggering expression of novel proteins and secondary infarct expansion (Kleinig and Vink, 2009). Innate and adaptive immune systems participate as well. At least early on, inflammation amplifies the ischemic lesion, but, as discussed later, it may set the stage for tissue repair in the late post-ischemic period. A number of therapeutic trials designed to target the inflammatory response have failed to show clinical benefit (Sughrue, 2004), underscoring the need to clarify further the complexities of acute and delayed postischemic inflammatory signaling within the brain. As noted above, cerebral blood vessels are the first to be exposed to the ischemic insult, and their reaction to injury sets the stage for the inflammatory response. Early on, production of cytokines, such as TNF- α and IL1b, in vascular cells and perivascular microglia-macrophages upregulates adhesion molecule expression (e.g., ICAM-1, P and E-selectin) and, along with integrins, promote leukocyte rolling and sticking to the vessel surfaces (Zhang 1998). Neutrophils are the first blood-borne cells to be recruited into the brain, followed by monocytes and, starting on days 1–2, lymphocytes. As the brain's primary immune cell, activated microglia transform into macrophages and accumulate at the border zone along with blood-borne macrophages to clear debris and dead cells and produce pro-inflammatory mediators and toxic molecules (Schilling, 2003). Molecules released from injured or dying brain cells also contribute to the inflammatory response. In particular, products from cells undergoing oxidative stress and necrosis, for example lipopeptides, advanced glycation end-products (AGE), modified lipids, heat shock proteins, hyaluronic acid, and the nuclear protein HMGB1, lead to activation of the innate immune system by engaging toll-like receptors (TLRs). TLRs are expressed in multiple cells and, in conjunction with CD36, receptors for AGE (RAGE), and other scavenger receptors, may serve to detect “danger signals” triggering NF κ b-dependent inflammatory signaling (Oppenheim and Yang, 2005; Wang, 2007). PARP activity appears to function as an NF κ b coactivator (Chiarugi and Moskowitz, 2003), with therapeutic potential

for delayed administration of PARP-1 inhibitors (Kauppinen, 2009). Besides NF- κ B, other transcriptional activators, including activator protein-1, STAT-3, interferon regulatory factor 1, and C/EBP beta, exert a proinflammatory effect (Kapadia, 2006; Iadecola, 1999), while PPAR γ acts as a negative modulator of postischemic inflammation (Kapadia, 2008). In addition to neutrophils and monocytes, T cell subsets have recently been implicated as modulators of secondary infarct progression. Treg cells express the antiinflammatory cytokines IL10 and TGF- β and normally dampen the immune response by suppressing T cell effector activity (Liesz, 2009). Secondary infarct progression is impacted by a second subset of invading T lymphocytes, gamma-delta T cells, a source of the proinflammatory cytokine IL-17. After deletion of IL-17, infarct size decreases (Shichita, 2009).

These findings support the notion that taming post-ischemic inflammation may block secondary events that extend brain injury. However, as noted below, during the later stages in the injury process, inflammation promotes critical events necessary for tissue repair. Therefore, therapeutic interventions targeting post-ischemic inflammation should be mindful of temporal considerations by minimizing the destructive potential of inflammation in the acute phase while enhancing its beneficial contributions to tissue repair in the late stages of cerebral ischemia. Necrosis, Necroptosis, and Autophagy: The Execution Necrosis and apoptosis are the principal mechanisms of cell death after ischemic injury, and the details of both have been mentioned (Yuan, 2006, 2009). Depending in part upon the magnitude, type of stimulus, and cell type, a number of cell death triggers promote necrosis or apoptosis, although cellular features of apoptosis and necrosis are sometimes found together in dying ischemic cells. Probably due to its ATP dependence, apoptotic cell death is prominent after milder injury and is more delayed (Bonfoco, 1995), although severe ischemia is associated with early-onset of caspase cleavage, enzyme activation, and acute cleavage of substrate proteins (Namura et al., 1998). Apoptosis and necrosis can be induced by multiple triggers, such as Ca²⁺ overload or oxidative stress. Mitochondria play an essential role in both (Schinzel, 2005), serving as a reservoir for proapoptotic and antiapoptotic proteins and cytochrome C. One such protein apoptosis-inducing factor (AIF) is released from the outer membrane of mitochondria in response to activation of the nuclear protein PARP and generation of a PAR polymer, its toxic product (Yu, 2006). AIF then translocates to the nucleus to promote a caspase-independent form of cell death, in part by promoting DNA fragmentation and chromatin condensation. Release of mitochondrial proteins sets in motion (or suppresses) multiple caspase-dependent and -independent processes to dismantle critical homeostatic and repair mechanisms. Despite the expression of executioner procaspases, activated caspases, and caspase cleavage products, classic apoptotic morphology is rarely found in humans within the adult ischemic brain. However, it does appear more prominently in the ischemic brains of neonates (Ferriero, 2004). Until now, necrotic cell death was defined as unregulated, unprogrammed, and lacking cell signaling events. It is the predominant form of ischemic cell death. It was recently shown that certain cells, including those within the brain, possess a molecular switch that determines whether cells die by necrosis or apoptosis in a TNF-dependent way (Holler, 2000). In this cell death variation, necrosis are triggered by two kinases, RIP 1 and RIP 3, reciprocally interacting, often in the presence of an apoptosis inhibitor (Cho, 2009). Activated RIP3 is capable of stimulating enzymes such as the glycogen-degrading enzyme, glycogen phosphorylase, to steer TNF-induced apoptosis toward necrosis, in part through bursting energy metabolism and ROS generation (Zhang, 2009). Nec-1, a small-molecule inhibitor of RIP-1, decreases ischemic cell injury in models of

ischemia-reperfusion and improves neurological outcome, suggesting RIP-1 as a potential therapeutic target (Degterev, 2005). Autophagy, an energy-dependent process implicated as a cell death mechanism, is used by eukaryotes to degrade and recycle subcellular organelles. Its precise role in the death process is controversial because autophagic activity may be cytoprotective, at least in some disease models, e.g., polyglutamine expansions or in aging models (Levine and Kroemer, 2009). In other disease models, such as in neonatal hypoxia-ischemia, autophagy reportedly promotes cell death (Puyal, 2009). Precisely how this is accomplished remains for further study.

Inflammation and Stroke

Currently there is increasing evidence that some form of inflammatory mechanism plays a role in the development and progression of stroke, especially in the setting of cerebral ischemia due to subarachnoid hemorrhage, head injury or cardiac arrest. It has been reported that low grade inflammation with raised levels of C-reactive protein (CRP) is an independent risk factor for stroke and TIA (Di Napoli M 2001). In pediatric population, inflammation without significant atherosclerosis has been associated with stroke. Novel imaging techniques have been adopted to facilitate *in vivo* observation of the dynamic effects of inflammation in causing brain tissue damage and neurologic outcome.

Inflammatory conditions associated with stroke. Stroke has been associated with a wide range of systemic inflammatory conditions. It may be associated with primary vasculitis, like giant cell arteritis, primary angitis of CNS, Takayasu's arteritis; vasculitis secondary to SLE, progressive systemic sclerosis, rheumatoid arthritis; and other inflammatory conditions like inflammatory bowel disease (IBD) and sarcoidosis. Fortunately the majority of these are rare, with stroke being an unusual complication in most cases (Emsley HC 2002, Hu Z 2000).

Infective Conditions Associated with Stroke

A number of acute and chronic infective conditions have been observed to precede a stroke. Most often these are of respiratory and bacterial origin, occurring within the previous month. These cases tend to present with more neurological deficit as compared to ones without infection (Campbell LA 2000, Grayston JT 1990, Tarnacka B 2002). Of the chronic infections *Chlamydia pneumoniae* has been known to affect macrophages/monocytes, endothelial cells, and vascular endothelium and thus having a pivotal role in atherogenesis. Evidence to this regards have been in the form of demonstration of the organism in atherosclerotic plaques of coronary artery and middle cerebral artery (Campbell LA 2000), and seropositivity in more than 50% individuals in their sixth decade (Grayston JT 1990). Currently, an acute recrudescence of infection (diagnosed by immunoglobulin M titers) is associated with acute stroke and TIAs. Similarly, increased serum levels of immune complexes containing chlamydial lipopolysaccharide have also been associated with increased stroke incidence and worse clinical outcome (Tarnacka B 2002). *Helicobacter pylori* have also been implicated in stroke pathogenesis, with one report emphasizing association with small vessel occlusion. However, seropositivity for *H. pylori* may be an independent risk factor for ischemic stroke (Emsley HC 2002, Grau AG 2001). Recurrent or

chronic respiratory infection (independent from acute episodes), herpes virus, periodontal infection (gingivitis or periodontitis) has been suggested to be independent risk factors in ischemic stroke (Emsley HC 2002, del Zepo G 2000). Neurological complications, though, are common in human immunodeficiency virus infections, associated with stroke remains unclear. It has been suggested to conduct randomized intervention studies, especially in cases of *C. pneumoniae*, in order to resolve the uncertainty over the temporal sequence of infection and stroke.

Inflammatory Mediators in Acute Stroke

The onset of ischemic cascade heralds the initiation of various processes, including inflammation, excitotoxicity, nitric oxide production, free radical damage, and apoptosis, all of which play a role in tissue injury. The molecular consequences of brain ischemia include temporal changes in cell signaling, signal transduction, metabolism, and gene regulation/expression. Currently these are potential therapeutic targets to minimize tissue loss and neurologic deficit by lessening the proportion of penumbral tissue recruited into the infarcted core. Inflammation occurs both by the molecular and cellular components at the blood-microvascular endothelial cell interface (Tan asesco 2008, Adibhatla 2008). With the onset of brain ischemia, microglia, astrocytes, endothelial cells, and neurons are known to release a number of cytokines, like interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), which enables leukocyte recruitment, activation, and the final adhesion to the endothelium of the cerebral microvasculature. These activated leukocytes obstruct the vascular channel, followed by transmigration in the infected areas along with monocytes/macrophages. Similarly, reperfusion, and systemic inflammatory processes, both before and after acute stroke, may also stimulate the inflammatory response, thus hampering the efficacy of thrombolytic therapy.

Inflammatory Gene Expression

Cerebral ischemia is known to upregulate the expression of a number of proinflammatory genes like transcription factors, heat shock proteins, cytokines, chemokines, and adhesion molecules. Among this transcription factor, nuclear factor (NF)- κ B, has been demonstrated to regulate expression of TNF- α , IL-1 β , IL-6, nitric oxide synthase (NOS), cyclooxygenase-2 (COX-2) and intercellular adhesion molecule-1 (ICAM-1) during *in vitro* conditions (Tan asesco 2008, Adibhatla 2008, del Zoppo G 2000). In the periphery (NF) - κ B, was expressed by activating macrophages, monocytes, lymphocytes, endothelial cells, fibroblasts, platelets, while in cerebral infarctions (NF)- κ B has been seen to be induced in activated microglia and glial cells (Terai K 1996).

Cytokines

These are polypeptides associated with inflammation, immune activation, and cell differentiation or death. In systemic setting these are produced by activating macrophages, monocytes, lymphocytes, endothelial cells, fibroblasts, platelets, and many other cell types,

whereas in CNS their source are activated microglia. Proinflammatory cytokines like TNF α , IL-1 β lead to parenchymal damage, while IL-10 and IL-1 receptor antagonist (IL-1ra) have an anti-inflammatory and neuroprotective role.

- *Tumor necrosis factor- α* : Permanent or focal MCAO has been associated with induction of TNF- α mRNA. The effects of this proinflammatory cytokine have been controlled by administration of soluble TNF-receptor 1 (sTNF-R1) or anti-TNF- α monoclonal antibody. The TNF - α expression appears sequentially in ischemic core, periinfarct areas, adjacent brain, followed by remote areas. CSF and blood levels have been elevated in most ischemic stroke patients, within first 6–12 and 24 h of onset. In lacunar infarcts, elevated levels have been associated with early neurologic deterioration and poor functional outcome. Elevated sTNF-RI and sTNF-RII concentrations are associated with carotid atherosclerosis, while sTNF-RI concentrations are increased in patients with acute ischemic stroke and infection (Adibhatla RM 2008, Emsley HC 2002, Buttini M 1996, Elkind MS 2002).
- *Interleukin-1*: Experimental studies have proved its proinflammatory property, which have been neutralized by IL-1ra administration (Emsley HC 2005). In contrast, neutralizing antibody to IL-1ra has resulted in enhancing the effects of brain ischemia. Increased IL-1 messenger RNA (mRNA) expression in peripheral mononuclear cells have been seen 1–3 days after onset of symptoms and reducing to normal levels by 20–31 days (Kostulas N 1999). Elevated plasma IL-1ra levels have been seen in patients with acute ischemic stroke within 4 $\pm$ 2 days (Beamer NB 1995, Emsley HC 2002, Adibhatla RM 2008).
- *Interleukin-6*: Cerebral ischemia is a potential bioactivator of IL-6 mRNA, especially in middle cerebral artery occlusion (MCAO) in animal models. Intracerebro-ventricular injection of anti-inflammatory IL-6 has been associated with significant reduction in ischemic damage. In a clinical study, circulating IL-6 levels were found to increase significantly, reaching a plateau between 10 h and 3 days, before returning to baseline by 7 days. It also correlated with volume of computed tomography of brain lesion, as well as, poor functional and neurologic outcome. Similar correlation in CSF studies has also been noted. However, IL-6 may also have a proinflammatory role, as in advanced atherosclerosis.
- *Transforming growth factor- β* : Increased expression of TGF- β 1 RNA and protein expression has been seen after an ischemic stroke, especially in infarct border zones (Krupinski J 1996). Its expression in penumbra and in recovery phase of some CNS diseases suggests a neuroprotective function.
- *Interleukin-10*: In ischemic and hemorrhagic stroke cases, peripheral blood mononuclear cells are seen to secrete this neuroprotective cytokine (Van Exel E 2002). Studies have shown that lower IL-10 levels are associated with increased risk of stroke.
- *High mobility group protein-1 (HMG-1)*: It is a ubiquitous and abundant nuclear and cytoplasmic protein and a chromatin component in eukaryotic cells; which acts by binding to DNA, in addition to facilitating gene transcription and stabilizing the nucleosome structure. It is released and secreted by activated innate immune cells,

macrophages, monocytes and astrocytes, and can act as a proinflammatory mediator, and also acts to promote the release of other proinflammatory mediators (Kumar A 2008). Experimentally antiHMG1 antibodies have been used to counter its effect on ischemic injury.

- *Other cytokines*: Limited studies and evidence are available with interferon- γ (IFN- γ), IL-2, IL-4, IL-16, IL-17, and granulocyte-macrophage colony stimulating factor (GM-CSF) (Emsley HC 2002).

Chemokines

These polypeptides are generally smaller than cytokines, and have a role in cellular communication and inflammatory cell recruitment in host defense. Of these, IL-8 and monocyte chemo-attractant protein-1 (MCP-1), of the CXC and CC chemokine families, respectively, have been implicated in cerebral ischemia (Mennicken F 1999):

- *IL-8*: Studies have noted that patients with ischemic stroke have IL-8 mRNA expressing mononuclear cells in blood and elevated IL-8 in plasma, which possibly has a proinflammatory role in recruiting neutrophils. Overall, CSF levels are higher than plasma level, indicating its site of production in CNS (Kostulas N 1999). Interestingly, CSF levels of IL-8 tend to increase initially and then fall rapidly in large infarcts or gray matter infarcts suggesting a proinflammatory role, in contrast to constant elevated levels (for 3 months) in small white matter lesions suggesting a neuroprotective function (Tarkowski E 1999).
- *MCP-1*: Significant rise in acute ischemic stroke (proinflammatory) has been associated with monocyte/ macrophage infiltration (Losy J 2001).
- *Interferon- γ -inducible protein-10 (IP-10)*: In a recent study by Canoui-Poittrine et al. (Canoui Poittrine F 2009), it was observed that in healthy middle-aged men, higher systemic levels of chemokines like IP-10 and RANTES are most likely independent predictors of future ischemic stroke.
- *CC chemokine ligand-5 (CCL-5)/Regulated on Activation Normal T Cell Expressed and Secreted (RANTES)*: Aggregates of RANTES, a member of the CC chemokine family, that form on the cell surfaces have been reported to mediate through its receptor (CCR5), and act as powerful activators of leukocytes for promoting the directed migration of leukocytes into damaged or inflamed tissue (Appay V 2001, Backon KB 1995, Canoui Poittrine F 2009). It is produced by a variety of cells, including T-lymphocytes, platelets, endothelial cells, smooth muscle cells, and glial cells. A study by Terao et al. (Terao S 2008) strongly implicated this chemokine in the pathogenesis of experimental ischemic stroke, and suggested that the RANTES that mediates the cerebral inflammation, blood–brain barrier dysfunction, and tissue infarction is largely derived from circulating blood cells.

Nitric oxide and cyclooxygenase-2 (COX-2) Three NOS isoforms exist (Del Zoppo G 2000):

- Neuronal NOS: Harmful effects on ischemic stroke.

- Endothelial NOS: Beneficial effect, since it causes vasodilatation.
- Inducible or immunologic NOS (iNOS): Contributes to ischemic injury. Inhibition of iNOS reduces infarct volume in middle cerebral artery obstruction (MCAO), and it has been demonstrated in neutrophils and blood vessels in patients who died within 24 h of ischemic stroke (Forester C 1999).

Experimental cerebral ischemia have shown elevated COX-2 mRNA and protein, as well as toxic prostanoids and reactive oxygen species, secreted by microglia, at the border of the ischemic territory, and thought to exacerbate the effects of ischemia (del Zoppo G 2000).

Matrix Metalloproteinases (MMPs)

These are a family of proteolytic enzymes involved in extracellular matrix modeling, but may contribute to the neuroinflammatory response. Upregulation of MMP-9 in the acute phase, as compared to MMP-2 in chronic recovery stage reflects both deleterious and beneficial effects (Adibhatla RM 2008, Mun-Bryce S 1998). MMP, especially MMP-9 levels, have been associated with poor neurological outcome, infarct growth, and hemorrhagic transformation events (Montanner J 2001 and 2003, Ross A 2008).

Adhesion Molecules

Leukocyte interaction with vascular endothelium is mediated by three main groups of cell adhesion molecules: selectins (comprising P-selectin, E-selectin, and L-selectin); integrins (e.g., lymphocyte function-associated antigen-1, and Mac-1); and the immunoglobulin superfamily (which includes ICAM-1 and vascular adhesion molecule-1: VCAM-1) (DeGraba TJ 1998). These molecules not only enable leukocyte infiltration during acute stroke, but also have a role in pathogenesis of atherosclerosis and changes in atheromatous plaque. In the human brain, the expression of ICAM-1, VCAM-1, and E-selection of cerebral microvascular endothelial cells is increased by IL-1 β , TNF- α , and lipopolysaccharide. Localized ICAM-1 type is expressed in histologically normal human carotid bifurcation, a high-risk region for the development of atherosclerotic plaque, whereas endothelial ICAM-1 expression is increased in symptomatic versus asymptomatic carotid plaque. Serum levels of sICAM-1 (and E-selectin) are elevated in cerebrovascular (large and small vessel) diseases; becomes high after cerebral ischemia; and attains peak levels in 24 h of acute ischemic stroke. In contrast, sVCAM-1 elevates between days 1 and 5, and peaks at 5 days (Emsley HC 2002).

Neuropeptides

A number of neuropeptides have been implicated in the genesis of neurogenic inflammation, including substance P (SP), calcitonin gene-related protein (CGRP) and neurokinin A (Karaszkeski B 2009). Stimulation of the neuronal C-fibers by vanilloids, histamine or prostaglandins, among others, leads to neuropeptide release.

- *Neuropeptide Y (NPY)*: This modulates the immune cell distribution, T helper cell differentiation and natural killer cell activation. Studies have shown that NPY and its receptor Y1 partly mediate ischemic/reperfusion injury via upregulation of nNOS and eNOS, the effects of which can be controlled by NPY-Y1 receptor inhibition. NPY levels, in experimental studies, were found to be increased at 6 h in the peri-

ischemic region and peaked at around 3 days, decreasing at 10 days (Askalan R 2001).

- *Substance P*: Most potent of the neuropeptides. Is a member of the tachykinin family that also includes neurokinin A (NKA), neurokinin B (NKB) and neurokinin? (NK?) (Turner R 2007). These peptides are produced from preprotachykinin genes 1 and 2 (Campos MM 2000) SP potentially binds to three receptors (NK1, NK2, NK3) but has a higher affinity for the neurokinin 1 (NK1) receptor, which results in neurogenic inflammation, manifesting as increased vascular permeability, vasodilatation, tissue swelling and cell migration. Release of SP maybe one of the earliest pathophysiological events associated with injury to the brain, leading to release of inflammatory cytokines, free radical and endothelial nitric oxide (Persson MG 1991). SP is capable of regulating the action of other neurotransmitters, including dopamine and acetylcholine, and the opening of inward cation channels, in particular calcium. Levels of SP has been observed to be raised in ischemic stroke, while administration of an SP antagonist (SR-140333, nelpitantium besilate) significantly reduced infarct volume and improved neurological function measured at 24 h following ischemia (Karaszkeski B 2009).

Cellular Arm

The key components of this arm are blood derived leukocytes, which have a role in secondary brain damage in ischemic and reperfusion stages; while microglial cells, are the major CNS source of cytokines and other mediators, and also become phagocytes when fully activated by neuronal death.

Leukocytes

Elevated total leucocyte counts, independent of smoking, have been associated with higher incidence of vascular diseases and cerebral infarction. Various studies have shown that neutrophil accumulation starts within 6–12 h of onset, increases for 24 h, peaks in 48–72 h, and finally replaced by mononuclear cells in 4–6 days. This also correlates with CSF neutrophil levels (Akopoy SE 2006).

Microglia

Generally microglia is in a resting phase and constitutes 5% to 20% of glial population. During ischemia, depending on the time and extent, these get activated, and undergo substantial morphological and metabolic transformation, along with rapid and profound genetic upregulation. Subsequently they transform into the principal CNS source of cytokines like IL-1 β , TNF- α , and TGF- β (Gregerson R 2000).

Acute Phase Reactants and Body Temperature

In the normal condition body tends to respond to various tissue injury, including inflammatory and infective conditions, by means of cytokines, primarily IL-6 and IL-1. The classic acute phase reactants in cerebrovascular ischemia are C-reactive proteins (CRP), serum amyloid A protein, and fibrinogen.

C-reactive Protein

Healthy person normally has a median CRP concentration of approximately 1 mg/L, while during any tissue injury, inflammation or infection it tends to undergo 100-fold or more increase. Elevated CRP levels have been demonstrated to predict the risk of: (a) first ischemic stroke among apparently healthy men; (b) future ischemic stroke and TIA in the elderly; and (c) fatal stroke in the elderly. Studies have shown a very early increase (within 3 h) in patients with acute stroke with or without infection, and are an independent predictor of survival or nonfatal vascular event after ischemic stroke (Hu Z 2000, Rost NS 2001).

Erythrocyte Sedimentation Rate And Fibrinogen (ESR)

In cases of ischemic stroke, elevated ESR is an independent predictor of poor outcome within the first month, and early stroke recurrence (Belestrino M 1998). In contrast, fibrinogen, is an independent risk factor for stroke and significant hyperfibrinogenemia occurs in patients with acute cerebral infarction in association with leukocytosis and an increase in leukocyte aggregation (D'Erasmus E 1991, Belch J 1998).

Body Temperature

Studies on animal models revealed hypothermia of the brain and body had profound influence on stroke outcome, since it tends to reduce infarct size, unlike hyperthermia, which enhances neuronal damage (Yenari M 2008, Mi'nembres E 2008). Subsequent clinical studies have shown that body temperature has been independently and significantly related to initial stroke severity, lesion size, mortality, and outcome in survivors. Elevated body temperature in patients with acute stroke, possibly due to infections, has been associated with increased morbidity and mortality.

Role of Neuroimaging in Evaluation of CNS Inflammation in Stroke

Newer imaging techniques have enabled *in vivo* evaluation of cerebral inflammation, and subsequent correlation with neurologic outcome and brain tissue damage. These include gamma camera imaging of reinjected 111indium-labeled leukocytes (Pozzili C 1985) demonstrating neutrophilic infiltration within 24 h of acute cerebral infarcts; and technetium-99m hexamethylpropyleneamine oxime (99mTc HMPAO)-labeled leukocyte brain single-photon emission computed tomography (Wang PY 1993), showing neutrophilic accumulation with infected areas. PK11195 is a specific ligand for the peripheral benzodiazepine binding site. After cerebral ischemia, increased binding of PK11195 is colocalized with invading the cells of mononuclear-phagocytic lineage, in and around infarcted tissue. Presence of activated microglia can be demonstrated for weeks after onset of ischemic injury (Gerhard A 2000).

Proposed Interactions Unifying Various Pathophysiologic Mechanisms

A wide range of mechanisms has been proposed for unifying and linking inflammation, infection, atherosclerosis and vascular risk factors in the pathogenesis of stroke.

Inflammatory Mechanisms

Apparently inflammation, marked by raising CRP levels, is the pivotal point, which not only contributes to atherogenesis, but also contributes to plaque rupture, precipitating acute thromboembolic phenomenon, culminating in acute stroke. However, the interactions between these multiple factors are quite complex. CRP may be elevated in a number of cardiovascular risk factors like increasing age, smoking, body mass index, lipid levels, and hypertension, while hypertension it may have a proinflammatory role mediated by sICAM-1 and IL-6 (Pasceri V 2000).

It may also be elevated in response to other infective etiologies, and have a proinflammatory effect by modulating expression of adhesion molecules on vascular endothelium, with subsequent recruitment of monocytes and other immune cells. These, along with activated T-cells release various growth factors and cytokines, like TNF- α and IL-1, leads to advanced phase of atherosclerosis (Barath P 1990, Galia J 1996).

Procoagulant State

A prothrombotic state in various inflammatory and infectious conditions, with altered immune function, predisposing the large vessel to atherothrombosis and stroke. Modulation occurs due to reduced circulating antithrombotic activated protein C (APC), elevated C4b-binding protein, and a low ratio of tissue plasminogen activator to plasminogen activator inhibitor, or due to increased fibrin D-dimer levels, Cardiolipin immunoreactivity, and fibrinogen levels (Kumar A 2008, Ameriso SF 19991).

Evidence of continued endothelial damage is reflected by increased levels of vWF, while acute episodes are represented by raising CRP levels, which act in tandem with proinflammatory cytokines, like IL-6, to produce a procoagulant state, which act by the extrinsic pathway (Di Napoli M 2001). Procoagulant state precipitating occlusive cerebrovascular disease is encountered in a number of disease process:

- (a) SLE: Antiphospholipid antibodies like anticardiolipin antibodies and lupus anticoagulant acts by inhibition of prostacyclin formation, causing platelet aggregation; inhibition of prekallikrein, alterations of antithrombin III, decreased fibrinolysis, decreased release of plasminogen activator, inhibition of protein C activation, and the induction of endothelial injury and thrombotic thrombocytopenic purpura (Kumar A 2008, Kitagawa Y 1990).
- (b) Inflammatory bowel disease: Affected by hypercoagulability-related thrombosis, vasculitis, and consumption coagulopathy leading to hemorrhagic events (Lossos A 1995).
- (c) Bacteriemia and septicemia: Incidence of thromboembolism in infective endocarditis and in septicemia is 20% and 10%, respectively. Mechanisms of thrombosis responsible include reduced levels of antithrombin III, proteins C and S, increased platelet aggregation and adhesion, impaired fibrinolysis, and antiphospholipid antibody formation; apart from the effects of endotoxins,

Other bacterial toxins, and proinflammatory cytokines such as IL-1 and TNF α (Veltonen V 1993).

Vasculitis and Altered Circulation

Though atherothrombosis and atherothrombotic embolism are the commonest mechanisms for cerebrovascular occlusion, other less frequent causes include inflammatory vasculitis, like giant cell arteritis, Takayasu arteritis and Wegener granulomatosis; and infective pathology due to *Mycoplasma* infection, varicella zoster and neurosyphilis (Emsley HC 2002, Eskalan R 2001).

Vascular supply may be compromised in cervical artery stenosis, due to preexisting anomalies of extracellular matrix; or due to cardioembolism in a setting of systemic lupus erythematosus (SLE) with valvular heart disease.

For the above considerations and more, there is a compelling need to accelerate efforts to interrogate the stroke process and define the links that exist with other conditions such as vascular and neurodegenerative dementia. It is also crucial to expand the narrow repertoire of therapeutic opportunities for these devastating conditions. To accomplish this, novel approaches are required that expand upon our evolving mechanistic understanding of the fundamentals of cell survival and death processes as well as tissue repair. The future depends upon how successful we are in deciphering these mechanisms and bringing clarity to the complex interactions between the multiplicity of cell and tissue types within the brain (Lo, 2003). Armed with this knowledge and its successful therapeutic application, the field of stroke could be transformed.

Keeping in view this spirit, we will now try to address the selected issues fundamental to the science of ischemic stroke and vascular dementia. It begins with posing questions about stroke risk factors followed by a discussion of key cell and tissue mechanisms that render brain susceptible as well as tolerant to ischemic injury, including those promoting tissue protection and repair. The chapter ends by highlighting promising treatment strategies, inspired by these endogenous mechanisms, which present the opportunity to open new avenues in stroke therapy.

Stroke Risk Factors and Triggers

A stroke risk factor is a characteristic of an individual that increases the risk for stroke compared to someone without that characteristic (Hankey, 2006). Some risk factors cannot be modified, such as a family history of cerebrovascular diseases, older age, male sex, and Hispanic or Black race (Allen and Bayraktutan, 2008; Hankey, 2006). Other risk factors are modifiable, and their correction reduces the chance of having a stroke (Table 1). These factors, which often coexist, have been estimated to account for 60%–80% of stroke risk in the general population (Allen and Bayraktutan, 2008; Hankey, 2006). Genome-wide association studies are increasingly being employed to identify susceptibility genes for stroke (Hegele and Dichgans, 2010). Although several loci have been identified, the need for independent replication and the modest effect sizes have precluded the full assessment of the clinical relevance of these findings (Hegele and Dichgans, 2010).

Table 1. Major Modifiable Risk Factors for Ischemic Stroke

Classification	Risk Factor	References
Casuals	Hypertension	Lawes et al., 2004a
	Diabetes	Lawes et al., 2004b
	Hypercholesterolemia	Amarenco et al., 2006
	cigarette smoking	Bonita et al., 1999
	atrial fibrillation	Hart et al., 1999
	valvular heart disease	Kizer et al., 2005
	ischemic cardiomyopathy	Loh et al., 1997
	carotid stenosis	Rothwell et al., 2003
Probable	Hyperhomocysteinemia	Wald et al., 2002
	increased inflammatory markers (WBC, CRP, infection)	Di Napoli et al., 2005
	elevated plasma fibrinogen	Rothwell et al., 2004
	Dyslipidemia	Holme et al., 2009
	patent foramen ovale	Nedeltchev et al., 2008
	obstructive sleep apnea	Culebras, 2009
	body mass index	Hu et al., 2007
	lack of exercise	Hu et al., 2005
	low fruit and vegetable diet	Dauchet et al., 2005
	psychosocial stress	Simons et al., 1998

^a Factors demonstrated in randomized clinical trials to reduce stroke risk if corrected, or for which a strong, consistent, and independent association with stroke has been established (Hankey, 2006).

^b Factors for which the association with stroke is less firm and/or no causative role has been established. WBC, white blood cells; CRP, C-reactive protein.

Risk Factors and Propensity to Stroke?

Risk factors have profound effects on the structure and function of blood vessels and on their interface with circulating blood. Many of the established risk factors alter the vascular structure by promoting atherosclerosis and stiffening of the arteries and by inducing narrowing, thickening, and tortuosity of arterioles and capillaries (Allen and Bayraktutan, 2008; Iadecola and Davisson, 2008). With the brain, these morphological changes are often associated with reductions in resting cerebral blood flow (CBF) and marked alterations in CBF regulation. Thus, aging, hypertension, diabetes, and hypercholesterolemia impair vital adaptive mechanisms that assure that the brain is adequately perfused (Arrick, 2007; Iadecola and Davisson, 2008; Iadecola, 2009; Kitayama, 2007). The ability of the endothelium to regulate microvascular flow is compromised, while the increase in blood flow evoked by neural activity is suppressed, resulting in a mismatch between the brain's energy supply and demand (Arrick, 2007; Iadecola and Davisson, 2008; Iadecola, 2009; Zou, 2004a).

Some risk factors, like hypertension and diabetes, impair protective vascular mechanisms that keep CBF stable during reductions in blood pressure (cerebrovascular autoregulation), facilitating the occurrence of ischemia if intravascular pressure drops (Immink, 2004; Kim, 2008). These vascular alterations increase the brain's vulnerability to ischemia after arterial occlusion because they compromise the development of collateral flow arising from adjacent nonischemic vascular territories, which is vital to the survival of the ischemic

perinfarct zone (see Parenchymal Failure Section). In addition to their vascular effects, some risk factors, like aging and diabetes, may enhance the intrinsic susceptibility of brain cells to injury, amplifying the tissue damage produced by ischemia (Biessels, 2002), but the biological bases of this effect are not well understood. Little is known about the interaction among the different stroke risk factors and whether their vascular effects are additive or synergistic. Furthermore, the relative contribution of parenchymal and vascular factors to stroke risk remains to be determined.

Cerebral Blood Vessel Alteration by Risk Factors?

Many cardiovascular risk factors increase the production of reactive oxygen species (ROS) and promote inflammation in systemic and cerebral blood vessels. The predominant vascular sources of ROS are the superoxide-producing enzyme NADPH oxidase, xanthine oxidase, mitochondrial enzymes, and uncoupling of nitric oxide synthase (NOS), a state in which this enzyme generates superoxide instead of NO (Faraci, 2006) (Figure 4).

Many of the damaging effects of oxidative stress on blood vessels are related to the biological inactivation of NO by the free radical superoxide, which reduces NO bioavailability and prevents its beneficial effects (Pacher, 2007; Schulz, 2008). Thus, loss of the vasoregulatory effects of endothelial NO leads to vasoconstriction and reduced NO-dependent vascular responses, with a negative impact on the regulation of microvascular flow (Schulz, 2008). Loss of the antiaggregant, antiproliferative, and anti-cell adhesion effects of NO promotes platelet aggregation, leukocyte adhesion to endothelial cells, and smooth muscle proliferation, key steps in vascular inflammation (Pepine, 2009; Zou, 2004b).

In addition, ROS can directly promote inflammation by increasing blood-brain barrier (BBB) permeability through upregulation of vascular endothelial growth factor (VEGF) and by inducing the expression of cytokines, matrix remodeling enzymes, including metalloproteases, and proinflammatory genes through NF κ B activation (Marchesi, 2008). One such NF κ B-dependent gene product, inducible nitric oxide synthase, produces large amounts of NO, which alter vascular structure and function through nitration and nitrosylation of critical proteins (Gunnnett, 2005; Guzik, 2003; Lima, 2010).

Peroxynitrite, a potent nitrating agent produced by the reaction of NO with superoxide, alters vasomotor reactivity by inactivating critical endothelial and smooth muscle enzymes and by activating the DNA repair enzyme poly-ADP-ribose polymerase (PARP), which leads to ATP depletion and vascular ion channel dysfunction (Pacher, 2007). Whereas ROS may set the stage for inflammation, vascular inflammation, in turn, leads to ROS production, creating a vicious circle that enhances vascular damage. Activation of the plasminogen system by oxidative stress and inflammation promotes matrix remodeling, smooth muscle cell migration, and intimal hyperplasia (Nicholl, 2006), factors that promote atherosclerosis and alterations in vascular structure. Thus, oxidative stress and vascular inflammation are major pathways through which risk factors exert their deleterious effects on blood vessels. However, it remains to be determined how individual risk factors trigger the activation of one or both of these processes. This is a critical question for targeting preventive strategies in patients with specific risk factors.

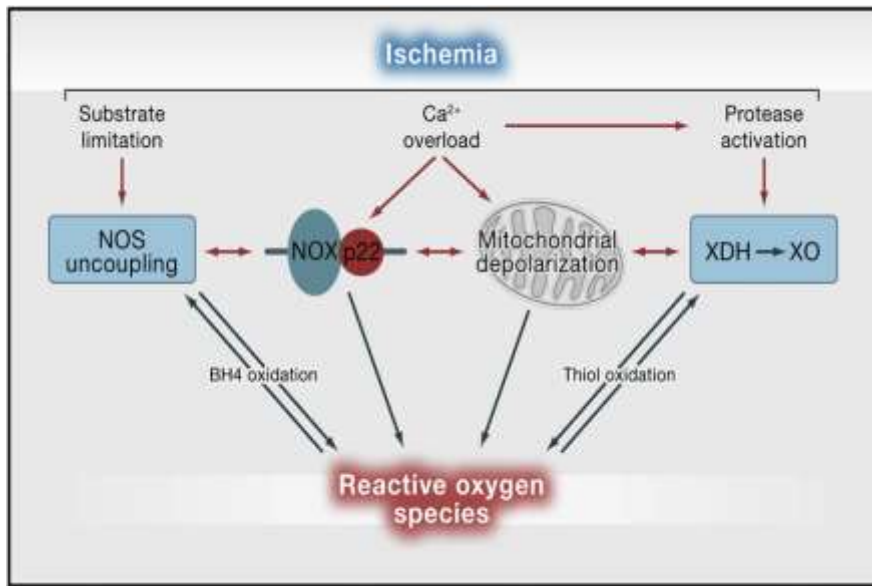


Figure4. Major Sources of ROS in Brain and Blood Vessels. Stroke risk factors and ischemia lead to the activation of several ROS-generating enzymatic systems. In ischemia, the increase in cytosolic Ca^{2+} activates the superoxide-producing enzyme NADPH oxidase (NOX), through PKC and NO derived from neuronal NOS (Brennan,2009; Girouard,2009). Mitochondria become depolarized by the Ca^{2+} overload (Nicholls, 2008) and, possibly, by ROS derived from NADPH located near the mitochondria (Girouard, 2009) producing large amounts of superoxide. The Ca^{2+} -induced activation of proteases and/or the pro-oxidant environment convert xanthine dehydrogenase (XDH) to xanthine oxidase (XO) (Abramov,2007), a superoxide-generating enzyme involved in purine degradation. Finally, the substrate deprivation induced by ischemia and the oxidative inactivation of essential cofactors like tetrahydrobiopterin (BH4) uncouple L-arginine oxidation from NO formation by NOS, resulting in superoxide production (NOS uncoupling) (Förstermann, 2010). Other potential sources of ROS include enzymes for arachidonic acid or catecholamine metabolism, but their participation in stroke-induced oxidative stress remains in question (Adibhatla and Hatcher, 2010; Kunz,2007a).

ROLE OF STROKE TRIGGERS

While vascular risk factors increase the likelihood of stroke in affected individuals, precipitating factors that act as “stroke triggers” must also be postulated. In some patients, the specific event responsible for the arterial occlusion can be identified, e.g., neck trauma leading to dissection and occlusion of the Table 1. In most instances the factors precipitating the ischemic event cannot be established. Although systemic infections, pregnancy and puerperium, use of illicit drugs, and mental stress often trigger a stroke (Elkind, 2007), precisely how these factors exert their effect remains unclear. Exacerbation of vascular inflammation and activation of the coagulation cascade are likely to play a role (Welsh,2009). For example, the added vascular dysfunction and blood clotting abnormalities, superimposed on those induced by stroke risk factors, could precipitate vascular occlusion or hemodynamic insufficiency. This view is supported by the fact that acute stroke often occurs in the setting of increased circulating leukocytes and elevated plasma markers of systemic inflammation and vascular activation, which also predict a poor outcome (Elkind, 2007; Welsh,2009). Thus,

our understanding of stroke triggers is limited, and mechanistic studies addressing these issues would be valuable in the identification of high-risk cases.

Parenchymal Failure: Brain death during Ischemia?

Because of its high intrinsic metabolic activity and large concentrations of the neurotransmitter-excitotoxin glutamate (Choi, 1992), the brain is especially vulnerable to ischemic insult. This can develop either as a consequence of thrombosis in situ or following embolic occlusion of a cerebral blood vessel, the latter usually arising from the heart or atherosclerotic plaques in the carotid artery and aortic arch. Although neurological dysfunction occurs within seconds to minutes of vessel occlusion, the evolution of ischemic injury and cell death continues in stages for minutes, hours, and even days, depending, in part, upon the vulnerability of the particular brain region, its cellular constituents, and the extent of residual perfusion.

The responses of blood vessels and their perfusion are important to the outcome as well. For example, a deficiency of vasodilating molecules such as endothelial NO enlarges the stroke size (Huang, 1996). Early restoration of blood flow by clot lysis and reperfusion decreases ischemic injury, and this may be achievable by giving recombinant tissue plasminogen activator (tPA), the only FDA-approved treatment for reestablishing flow and salvaging brain tissue. However, tPA is used in <10% of patients and even less frequently after 3 hr because of the risk of hemorrhage into ischemic tissue (NINDS tPA Study Group, 1995). Moreover, tPA may have other unintended risks (Kaur, 2004), such as injury to the blood-brain barrier by activating matrix metalloproteinases (Wang, 2003) or excitotoxicity in experimental models (Nicole, 2001). Combination therapies that ameliorate these effects may extend the tPA's therapeutic window while mitigating the untoward effects of reperfusion and plasminogen activation (Liu, 2004; Cheng, 2006; Murata, 2008). Theoretically, that could prolong tissue viability for hours or even longer, depending upon the rapid delivery of drug to the compromised brain (Ginsberg, 2008). Whether or not the vulnerable brain tissue can be rescued or protected after a protracted time period in the absence of partial or complete restoration of blood flow is an unanswered question. In all likelihood, areas of vulnerable brain do remain viable for hours after vessel occlusion, at least in some instances. For example, the hemiparesis that follows middle cerebral artery occlusion in the baboon was reversed in 73% of animals with reperfusion at 2–4 hour, compared to 33% and 17% in animals with reperfusion at 8 and 16–24 hour, respectively (Marcoux, 1982; Crowell, 1981; Jones, 1981). The challenge then is to restore adequate blood flow quickly after vessel occlusion and to protect viable tissue from unleashing mechanisms that lead to cell and tissue demise.

Salvageable versus Non-salvageable Tissue as Target for Therapy: Tissue distal to an occluded blood vessel is often heterogeneous, meta-stable, and differentially susceptible to ischemic injury. This meta-stable zone, termed the ischemic penumbra or the perinfarct zone (Astrup, 1981), is characterized by significantly depressed tissue perfusion that is barely sufficient to support basal ATP levels and oxygen metabolism as well as normal ionic gradients in the presence of electrical silence and suppressed protein synthesis. The ischemic penumbra, then denotes an “at risk” region that is functionally impaired but potentially salvageable. However, unless perfusion is improved or cells made relatively more resistant to

injury, the tissue at risk dies within a few hours (Lo, 2008a, 2008b). The ischemic core by contrast, refers to the irreversibly damaged tissue distal to an occluded blood vessel, characterized by <20% of baseline blood flow levels, depleted ATP stores, and irreversible failure of energy metabolism (Lo, 2008b). With time, the infarct core expands into the ischemic penumbra, and therapeutic opportunity is lost. Therefore, detecting a penumbra in patients can help to identify those who might benefit most from acute treatments that restore blood flow (thrombolysis or endovascular clot retrieval) or treatments for the future that render viable brain more resistant to ischemic injury. For this purpose, magnetic resonance imaging methods such as perfusion-weighted and diffusion-weighted imaging are most often used. Perfusion-weighted imaging measures the spatial extent of blood flow compromise, whereas diffusion-weighted imaging is thought to detect the region of attenuated diffusion of water, a putative surrogate marker for severely damaged brain tissue. The difference is thought to reflect the ischemic penumbra (Figure 2) (Ebinger, 2009). Although the predictive value of this method is still debated, perfusion-diffusion mismatch provides a useful first attempt to define the tissue at risk.

Prominent Mechanisms Leading to Cell and Tissue Demise in Stroke?

Within the ischemic penumbra, multiple mechanisms have been identified over the past few decades that irreversibly damage brain tissue (Figure 5). Understanding the contribution of each informs us about potential therapeutic opportunities and treatment targets. In the area of severely limited blood supply, ATP utilization continues in the presence of minimal synthesis and levels drop, leading to acidosis and loss of ionic homeostasis. As a consequence, cells swell and membranes rupture. However, ischemic cell death cannot be equated with limited ATP availability; rather, tissue death develops as a consequence of numerous ionic, biochemical, and cellular events that impose overwhelming stresses upon already compromised tissue (Dirnagl, 1999; Lo, 2003).

Causes of White Matter Damage Underlying Vascular Cognitive Impairment VCI?

In the cerebral autosomal-dominant arteriopathy with subcortical infarct and leukoencephalopathy (CADASIL syndrome), a monogenic disease associated with dementia, extensive white matter damage is caused by notch-3 mutations in vascular smooth muscle leading to vascular insufficiency (Chabriat, 2009).

In common forms of lacunar disease, however, the mechanism of the white matter damage remains unclear, although family history, hypertension, diabetes, and chronic smoking are well established risk factors (Selnes and Vinters, 2006). The white matter lesions consist of demyelination, axonal loss, enlarged perivascular spaces, astrogliosis, and microglial activation (Fernando, 2006; Pantoni and Garcia, 1995). The wall of arterioles is thickened by accumulation of hyaline material (lipohyalinosis) or completely disrupted (fibrinoid necrosis), leading to microhemorrhages (Fisher, 1968; Pantoni and Garcia, 1995). Capillary density, resting CBF, and cerebrovascular reactivity is reduced both in normal and affected white matter (Brown, 2007; Mandell, 2008; O'Sullivan, 2002).

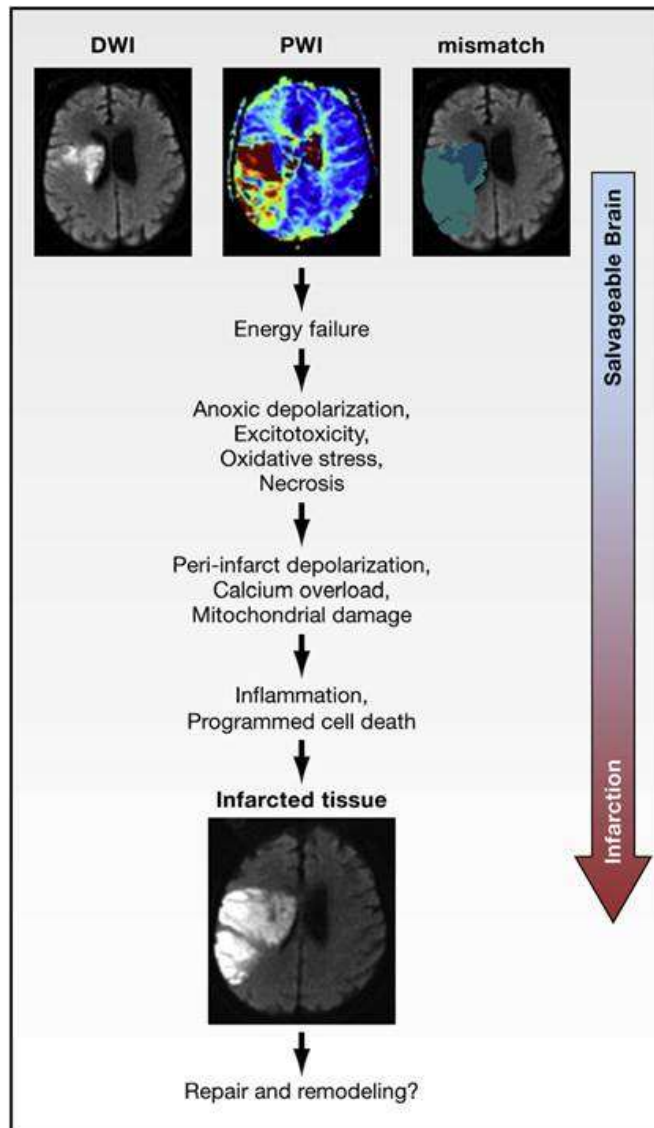


Figure 5. The Ischemic Penumbra. The ischemic penumbra represents compromised, but viable brain tissue lying distal to an occluded blood vessel. The top three MR images in this figure were taken in the same patient early after occlusion of the middle cerebral artery. The images are shown in the horizontal plane passing through the lateral ventricles. “Stunned, but not dead tissue” is detected by magnetic resonance imaging (MRI) techniques that assess (1) the spatial extent of the perfusion or blood flow deficit that presents as red and yellow areas on perfusion-weighted imaging (PWI, top middle image) and (2) severely damaged tissue in the ischemic core that presents as high-intensity areas on diffusion-weighted imaging (DWI, top left image) (Ebinger, 2009). In this patient, the perfusion deficit is larger than diffuse lesion. The top right image depicts the mismatch between core areas with a diffusion lesion (dark blue) and the larger areas with low perfusion (light blue-green). Over hours to days (see text), the core territory expands due to the complex cascades involving excitotoxicity, oxidative stress, programmed cell death mechanisms, and inflammation, as the initial trigger of energy failure ultimately progresses to infarcted tissue. The resulting infarct is shown in the image at the bottom of the figure and is about the size of the initial perfusion deficit in this untreated patient. In later stages, some patients may also partially recover, in part due to endogenous mechanisms of repair and remodeling (Chopp, 2007). MRI scans courtesy of G. Albers, Stanford.

Disruption of the arteriolar wall by amyloid, as observed in genetic or sporadic forms of cerebral amyloid angiopathy and in AD, is also associated with white matter lesions (Weller, 2009). Hypoxia-inducible genes and markers of endothelial activation are unregulated, suggesting hypoxia-ischemia (Fernando, 2006).

A causative role of hypoxia-ischemia is also suggested by the fact that the blood supply to the subcortical and basal ganglia white matter is provided by terminal arterioles located on the watershed between two vascular territories: perforating arteries from the neocortex and penetrating branches arising from larger arteries at the base of the brain (Iadecola, 2009). Therefore, these areas may be particularly susceptible to vascular insufficiency, especially if the structure of the arterioles and their vasomotor function are altered. On the other hand, energy and blood flow requirements of white matter are much lower than those of gray matter, rendering the tissue, in theory, less vulnerable to anorexia-ischemia (Hertz, 2008). Therefore, alternative mechanisms leading to an increased intrinsic vulnerability to injury may also play a role. For example, risk factors for dementia could enhance white matter susceptibility to anorexia, as aging has been described to do (Baltan et al., 2008). Disruption of the BBB, an early event in leukoaraiosis, could lead to injury by producing perivascular edema, microhemorrhages, and inflammation, contributing to demyelination (Farrell and Wardlaw, 2009). Demyelinated axons may be more susceptible to ischemic injury due to their heightened metabolic requirements caused by loss of energy-efficient salutatory conduction and leaky sodium channels (Trapp and Stys, 2009). Furthermore, dysfunction within the endothelium may compromise the trophic effect that these cells exert on neurons and glia, contributing to brain dysfunction and damage (see section on repair mechanisms). Aging, inflammation, hypoxia and, possibly, other risk factors can also reduce the repair potential of the damaged white matter by impairing oligodendrocyte progenitor recruitment and differentiation (Back, 2002; Pang, 2010; Sim, 2002). Therefore, the mechanisms underlying leukoaraiosis might be distinct from those of ischemic cell death and are a fertile ground for new investigations.

Stroke and Dementia?

The major mechanisms of tissue dysfunction and death cited above develop as a consequence of acute vessel occlusion and the attendant activation of endogenous mechanisms of cell injury and tissue demise. More subtle and less well understood mechanisms are beginning to define a new research frontier linking cerebrovascular dysfunction and small strokes to the development of vascular cognitive impairment (VCI) and Alzheimer's disease (AD), the most common causes of dementia (Fotuhi, 2009). AD and "vascular" dementia have traditionally been considered distinct entities (Iadecola, 2004). Thus, AD, the most prevalent of the two, is characterized pathologically by deposition of the amyloid β peptide (Ab) in the brain parenchyma (amyloid plaques) and blood vessels (amyloid angiopathy) and by neurofibrillary tangles (Querfurth and LaFerla, 2010). On the other hand, cerebrovascular diseases can lead to cognitive impairment in different ways. For example, a single stroke can cause dementia by damaging brain regions critical for cognition (strategic infarct dementia), while multiple strokes can cause stepwise cognitive deterioration through cumulative brain damage (multi-infarct dementia) (Leys, 2005; Pinkston, 2009). Most often, VCI has been due to small white matter lesions (leukoaraiosis) that are thought to

interrupt neural pathways involved in cognition (Pinkston, 2009). A recent realization has been that AD and cerebrovascular diseases coexist in up to 60% of cases (Leys, 2005; Pinkston, 2009). Thus, although cases of “pure” AD and vascular dementia can exist, in most cases the cognitive decline can be attributed to overlapping cerebrovascular and AD pathologies (Querfurth and La-Ferla, 2010). The causes of leukoaraiosis and the overlap between AD and cerebrovascular diseases are discussed in the next sections. Stroke doubles the risk of developing dementia (Leys et al., 2005). Although, as discussed above, location, size, and number of ischemic lesions, as well as leukoaraiosis can account for the cognitive impairment in some cases, epidemiological and pathological evidence indicates that stroke also increases the risk for AD. Alzheimer pathology can facilitate the development of dementia in patients with ischemic injury, and, vice versa, ischemic injury can aggravate the cognitive deficits induced by AD (Fotuhi, 2009). These findings have suggested a previously unrecognized interaction between amyloid pathology and ischemic injury. This is not surprising, considering that Ab has powerful cerebrovascular effects. Ab constricts cerebral vessels, suppresses vascular reactivity, and impairs autoregulation, leading to vascular insufficiency and increased susceptibility to ischemic injury (Iadecola, 2004; Zhang, 1997).

Conversely, ischemia and hypoxia can induce Ab accumulation by promoting its cleavage from APP (Tesco, 2007) and by downregulating the lipoprotein-related receptor protein-1, a critical receptor in the vascular clearance of Ab (Bell et al., 2009; Tesco, 2007; Wu, 2005). Therefore, in a vicious cycle, the vascular effects of Ab aggravate ischemia, and, in turn, brain ischemia enhances Ab accumulation (Iadecola, 2004). Recent studies have also linked plasma Ab to the white matter damage underlying leukoaraiosis (Gomis, 2009; Gurol, 2006). The significance of this finding and its underlying mechanisms remain to be defined and placed in context with other findings showing that Ab could promote axonal injury directly through its cytotoxic effects (Querfurth and LaFerla, 2010) or indirectly by inducing vasoconstriction and ischemia (Iadecola, 2004). Therefore, the vascular dysfunction induced by circulating and parenchymal Ab may act in concert with the structural and functional vascular changes induced by the risk factors to amplify the vascular insufficiency in small white matter arterioles leading to white matter injury. However, factors other than Ab could also contribute to the interplay between AD pathology and cerebrovascular diseases. For example, cerebral arterioles of AD patients exhibit a tendency to constrict due to increased activity of transcription factors (serum response factor/myocardin) that control the expression of contractile proteins in smooth muscle cells (Chow, 2007). This finding may play a role in the increased vasoconstrictor tone and reduced CBF observed in AD (Iadecola, 2004). It remains to be established whether these changes in gene expression are related to Ab or to upstream molecular events independent of it.

BRAIN REPAIRMENT AFTER STROKE

A well-known fact, in clinical stroke is that most patients show some degree of recovery over time. As the brain recovers, considerable reshaping of cortical areas takes place. Functional MRI studies demonstrate that peri-infarct areas are highly dynamic (Dijkhuizen, 2003; Chopp, 2007). Representational areas shift as latent networks are unmasked and parallel circuits are recruited adjacent to damaged regions (Murphy and Corbett, 2009). More

recently, advanced optical imaging techniques combined with Electrophysiology demonstrated that this remodeling at a circuit level can be correlated with specific processes of dendritic and synaptic plasticity at a cellular level (Li and Murphy, 2008). Imaging of mitochondrial function (Liu and Murphy, 2009) and vascular dynamics (Schaffer, 2006) has provided new insight into how the brain responds to microvascular perturbations following ischemia. These powerful tools may eventually allow one to interrogate mechanisms of stroke recovery at a molecular and cellular level while testing pro recovery therapies and strategies (Murphy and Corbett, 2009; Zhang and Chopp, 2009). Repair and remodeling processes after stroke; A major advance within this past decade has been the realization that adult mammalian brains also exhibit focal areas of ongoing neurogenesis in the subventricular zone and subgranular zone. Under normal conditions, these newborn neurons migrate to olfactory regions and hippocampus. But after cerebral ischemia or hemorrhage; the birth of new cells seem to increase and neuroblasts reroute toward damaged brain tissue (Arvidsson, 2002; Parent, 2002); perhaps as part of an endogenous attempt by the brain to repair itself. However, whether these new neurons significantly contribute to functional recovery remains unclear. Neurogenic responses may persist for surprisingly long periods of time, but the majority of these newborn cells survive only for a few days (Arvidsson, 2002). Whether these cells develop normally, express neurotransmitters, or even integrate into existing neural networks remains to be fully elucidated. Future attempts to promote endogenous neurogenesis for therapeutic purposes will have to address ways to modify parenchymal micro-environments to enhance neuronal survival and integrate newly born cells into viable circuitry. In concert with neurogenesis and neural plasticity the brain recovering from stroke exhibits complex patterns of vascular remodeling. Angiogenesis and vasculogenesis in perinfarct regions have been detected in rodent models of cerebral ischemia (Ding, 2008) as well as in human stroke (Krupinski, 1996). Indeed, it is now recognized that angiogenic and neurogenic responses are tightly coregulated after stroke and brain injury (Arai, 2009). This might not be surprising, because molecular mechanisms of neurogenesis and angiogenesis have been evolutionarily conserved so that similar mediators and pathways are involved in both phenomena (Carmeliet and Tessier-Lavigne, 2005). In the normal brain, the neurovascular niche defines these complex mechanisms of cell-cell signaling between cerebral endothelial and neural precursors in the subventricular and subgranular zones of ongoing neurogenesis. In the context of poststroke recovery, these close relationships between neurogenesis and angiogenesis are maintained. Neuroblasts migrate along perivascular routes (Thored et al., 2007). Promotion of neurogenesis enhances vascular regrowth and, conversely, angiogenic stimulation enhances neurogenesis (Ohab, 2006; Taguchi, 2004). Hence, it seems likely that brain recovery after stroke comprises interdependent neurovascular plasticity and remodeling processes that recruit multiple common mediators and signals (Snappyan, 2009). The new frontier of neurovascular repair may require a detailed exploration of how these multi-cell and multi-signal phenomena are regulated in the context of stroke. Recent experimental studies have suggested that pharmacological interventions such as EPO, statins, activated protein C, and phosphodiesterase inhibitors may promote functional recovery after cerebral ischemia (Wang, 2004; Zhang, 2006; Thiagarajan, 2008; Chen, 2009). Ultimately, therapies that can augment these endogenous signals and substrates of neurovascular remodeling may have long therapeutic time windows in stroke (Zhang and Chopp, 2009).

Implications for Therapeutic Intervention beyond Thrombolytics

Current protocols of primary stroke management and secondary prevention focuses on modifiable vascular risk factors such as hypertension, smoking, carotid stenosis, atrial fibrillation, physical inactivity, diabetes mellitus, and dyslipidemia, with usage of drugs like antiplatelet agents, anti-hypertensive drugs, lipid-lowering agents, and anticoagulant drugs. A recent addition to this armamentarium was intravenous tissue plasminogen activator in cases of acute ischemic stroke, the efficacy of which is often limited by stroke severity, older age, systolic hypertension, location of arterial occlusion, collateral blood supply, and time from stroke onset to treatment, and reperfusion-associated inflammation (Pandian JD 2009, Adibhatla RM 2008 and 2009). The overall re-canalization rate in thrombolytic therapy varies from 46.2% during the first 6–24 h of intravenous administration, to 63.2% in intraarterial and 83.6% with mechanical reperfusion techniques (Pandian JD 2009). Current understanding of various patho-genetic mechanisms of stroke has paved the path for newer therapeutic approaches.

Currently Available Agents with Anti-Inflammatory Role

Commonly used agents like the anti-platelet agent acetylsalicylic acid (aspirin) and the lipid-lowering agent's β -hydroxy- β -methylglutaryl coenzyme A reductase inhibitors (statins) possess anti-inflammatory effects in addition to their traditionally accepted actions.

Aspirin: Apart from its well-established role in prevention of death, myocardial infarction, and stroke in high-risk patients, aspirin has a direct role in modifying CRP levels, thus raising the possibility of an anti-inflammatory action apart from its *anti-platelet* effect mediated via COX inhibition. Based on the findings of the Second European Stroke Prevention Study (ESPS-2), and European/Australian Stroke Prevention in Reversible Ischemia Trial (ESPRIT); a combination of aspirin and extended-release. Dipyridamole was found superior to aspirin alone in reducing the occurrence of the primary combined end point of vascular death, nonfatal stroke, nonfatal myocardial infarction, and major bleeding complications, and found favor with the most recent American Heart Association guidelines. This was partly attributed to the anti-inflammatory actions of this combination therapy (Weyrich AS 2009). Further, it is also thought to block NF- κ B, which is the transcription factor for a host of proinflammatory mediators of ischemia. Since NF- κ B also has a role in the resolution of inflammation, excessive modulation might create a problem during the recovery phase (Grilli M 1996).

Statins: *Lipid-lowering effect* of statins has already established its efficacy by significantly reducing the incidence of ischemic stroke in patients with coronary artery disease, both with and without elevated serum cholesterol concentrations (Crouse JR 1998).

Anti-inflammatory and/or neuroprotective properties of statins: Statins have found its base in their ability to reduce CRP levels, especially ones with high CRP levels (Di Napoli M 2001). Its efficacy has been demonstrated by measuring the intimal–medial thickness on carotid ultrasound, an indicator of atherosclerotic disease. It has also been reported that statins achieve atherosclerotic plaque stabilization or even regression by reducing macrophage activation within the vessel wall, inhibition of MMP-induced plaque rupture, and prevention of cytokine-mediated smooth muscle cell proliferation, whereas reduction in serum cholesterol encourages an antithrombotic state, by reducing platelet aggregation or by lowering plasminogen activator inhibitor-1 levels (Mohler ER 1999). It also has a

neuroprotective action by upregulation of endothelial NOS and inhibition of iNOS, an effect associated with the augmented cerebral blood flow and reduced infarct size, reduction of leukocyte–endothelium interaction, modulation of CNS cytokine production, and antioxidant effects.

Recently, a Stroke Prevention by Aggressive Reduction in Cholesterol Levels study showed that treatment with high dose atorvastatin reduced risk of stroke in patients with recent stroke and transient ischemic attack and no known coronary artery disease (CAD) (Gedikli O 2008).

Angiotensin converting enzyme inhibitors and angiotensin II receptor blockers: The effects of these agents have been observed to reach beyond the known blood pressure lowering action mediated by the renin–angiotensin–aldosterone axis. Angiotensin II has proinflammatory effects via augmentation of expression of VCAM-1, MCP-1, and IL-6, and increased production of reactive oxygen species, which can be countered by angiotensin converting enzyme inhibitors or angiotensin II receptor blockers for having anti-inflammatory effect (Libby P 2001).

Novel Therapeutic Agents with Anti-Inflammatory Role

Neuroprotective agents: This includes a diverse range of drugs directed at restricting damage and salvaging the penumbral tissue. Though the small rim of penumbra acts as a barrier to the successful application of these drugs, their role can be vital in a setting where reperfusion obtained by combined thrombolysis and neuroprotective agent, is sufficient. These drugs act by modulating the excitatory amino acid system, controlling calcium influx, or can be metabolic activators, antiedema agents, inhibitors of leukocyte adhesion, and free radical scavengers. Unfortunately, despite the safety and efficacy being proved by more than 100 clinical trials its translation into clinical practice remains awaited (Lebs Kind DS 2001).

- **Free radical scavenger:** Edaravone is the first clinical drug for neuroprotection in the world which has been used from 2001 in most ischemic stroke patients in Japan, and is especially useful in thrombolytic therapy with tissue plasminogen activator (tPA) (Abe K 2008).
- **Gene therapy:** Neural stem cell (Abe K 2008) could provide a future regenerative potential against ischemic brain damage at chronic stage.
- **Inhibiting apoptotic mechanism:** Brain parenchyma under threat of infarction contains neuronal cells, which undergo caspase-dependant (either by the caspase-3-dependant intrinsic pathway, originating from mitochondrial release of cytochrome c; or caspase-8-dependant extrinsic pathway, originating by the activation of cell surface death receptors) apoptosis, as well as non-neuronal cells which undergo non-caspase-dependent apoptosis. Current studies are directed towards therapeutic intervention of these potentially salvageable areas (Broughton BR 2009, Yuwan J 2009).
- **Ischemic preconditioning and inhibition of oxygen sensors; Ischemic postconditioning:** The rationale behind this strategy is downregulation of cellular and tissue metabolism (Yenari M 2008), which is similar to that of hypothermia

(Minembres E 2008) to overcome the effects of ischemia. Further studies have described a new family of oxygen sensors—including prolyl hydroxylase domain-containing proteins 1-3 (PHD1-3)—which possibly has a role in balancing hypoxia tolerance, ischemic preconditioning and inflammation, and thus provide a novel setting for newer pharmacological interventions for ischemic and inflammatory diseases (Fraisl P 2009). Ischemic postconditioning was initially referred to a stuttering reperfusion and has been performed immediately after reperfusion, for preventing ischemia/reperfusion injury in both myocardial and cerebral infarction. It can be induced by a broad range of stimuli or triggers, and may even be performed as late as 6 h after focal ischemia and 2 days after transient global ischemia. Ischemic preconditioning or partial/gradual reperfusion, provides insights into the protective mechanisms of reperfusion injury and the acute, mitogen-activated protein kinase (MAPK), protein kinase C (PKC), and ATP-sensitive K⁺ (K(ATP)) channel cell signalling pathways (Edyinson L 2009, Tanahashi N 2009).

- **Hyperbaric oxygen therapy:** Hyperbaric (HBO) or normobaric oxygen (NBO) therapy applied in acute ischemic stroke aims to salvage irreversible tissue damage by supplying oxygen to the ischemic area. Oxygen delivered at higher pressures (2.5–3 ATA) within initial few hours appears more promising for bridging of a transient ischemic period until reperfusion of the penumbra takes place, and has shown to reduce infarct size from 30–40%. Recent studies have demonstrated that physical oxygen therapy indeed improves oxygen supply of the ischemic penumbra as well as the cellular bioenergetic metabolism. In this mechanism, the mitochondria, including their role in apoptotic cell death pathways as well as the modification of the cellular hypoxia sensor HIF-1 α are considered as potential “downstream pathways” of oxygen therapy (Poli S 2009).
- **Peroxisome proliferator-activated receptor-gamma (PPAR γ):** PPARs are ligand-activated transcription factors that control lipid and glucose metabolism. These have a role in gene expression, as well as may modulate gene transcription by directly interfering with other transcription factor pathways in a DNA-binding independent manner. Though all three isoforms (alpha, beta/delta, and gamma) are activated by the ischemic cascade, PPAR- γ , was shown to prevent post-ischemic inflammation and neuronal damage in several *in vitro* and *in vivo* models, and negatively regulating the expression of genes induced by ischemia/reperfusion (I/R). This may further be exploited for designing novel therapeutic targets (Collino M 2008).
- **Pituitary adenylate cyclase-activating polypeptide (PACAP) and vasoactive intestinal peptide (VIP):** Ischemic stroke not only leads to tissue degeneration, but also activates neuroprotective cellular mechanisms by releasing PACAP and VIP. These neuropeptides are widely distributed in both the central nervous system and peripheral organs of various vertebrates. They display pleiotropic biological activity and mediate via many intracellular pathways, with four categories of action: antiapoptotic, anti-inflammatory, metabolic, and modulation of gene expression. There are two structurally distinct receptors that recognize VIP peptides and PACAP with similar affinities (PACAP/VIPR-1, PACAP/VIPR-2), as well as a specific receptor for the PACAP peptide (PACAP-1). VIP has a wide physiological profile. In the periphery, VIP receptors are distributed throughout the gastrointestinal tract and genitourinary system, other smooth muscles and secretory glands and induce

relaxation in smooth muscle. VIP inhibits secretion in certain tissues, but stimulates secretion in others; and modulates activity of cells in the immune system.

In the CNS, VIP receptors are found abundantly in the cortex, hippocampus and thalamus, and have a range of both excitatory and inhibitory actions. All VIP receptors activate adenylyl cyclase (Wei Y 1996). Animal models confirm that the synthetic derivatives of these neuropeptides have considerable neuroprotective and anti-inflammatory potential, suggesting a possibility of their use as new therapeutic strategies in stroke management (Jo' zwaik-Bebenista M 2008).

- **Nuclear enzyme poly(ADP-ribose) polymerase 1 (PARP-1):** As discussed earlier, reactive oxygen and nitrogen species, including the reactive oxidant peroxynitrite, are generated in parenchymal, endothelial, and infiltrating inflammatory cells during ischemic stroke, and also during the reperfusion phase. These induce oxidative DNA damage and cause activation of the PARP enzyme family, which leads to depletion of its substrate NAD(+), slowing the rate of glycolysis, electron transport, and ATP formation, eventually leading to functional impairment or death of cells, as well as upregulation of various proinflammatory pathways. Animal model studies have shown significant therapeutic benefits of PARP inhibitors, which have entered clinical development for the experimental therapy (Pacher P 2008).
- **Induced hypothermia** is another strategy gaining popularity since it may limit the final extent of cerebral infarct. Though paracetamol has shown to decrease body temperature in acute stroke, however its efficacy needs further validation (Minnemans E 2008, Dippel DWJ 2001).
- **Minocycline in combination with tPA:** In an experimental study on spontaneously hypertensive rats, which were subjected to embolic focal ischemia using homologous clots and treated with combined minocycline at 4 h plus tPA at 6 h, it was noted that there was a decrease in plasma MMP-9 levels, reduction of infarction, and amelioration of brain hemorrhage. It was thus concluded that combination therapy with minocycline may extend tPA treatment time windows in ischemic stroke (Murata Y 2008).
- **Others:** Other therapeutic strategies are based on the neuroprotective action of erythropoietin (Maise K 2008), melatonin (Reiter RJ 2007), estrogen (Lebesgue D 2009), NOS inhibitors (del Zoppo G 2000, Bath PM 2002), and anti-high mobility group box 1 (HMGB1) monoclonal antibody (mAb) by altering permeability of the blood-brain barrier, expression of TNF- α , iNOS and MMP-9 (Mori M 2009).

Antimicrobial drugs and vaccines: Antichlamydial antibiotics also act by suppressing reactivation of chronic inflammation within atherosclerotic plaques, and have a potential role in secondary prevention (Grayston JT 1999). Influenza vaccine has been reported not only to reduce viral infection, but also secondary bacterial infections. A recent study has found a negative association between influenza vaccine and brain infarction, particularly in under 75 years population (Lavalley P 2002).

Antileucocyte agents: The deleterious effects of leucocytes in an ischemic setting have prompted the use of monoclonal antibody to competitively block its adhesion to ICAM-1, and thus limit the extent of damage, and also extend the therapeutic window, during which

thrombolytic therapy may be administered. Due to adverse outcome in the first human trial with anti-ICAM-1 murine monoclonal antibody, other strategies have been sought, viz. Antineutrophil serum and antineoplastic agents to achieve neutrophil depletion (Enlimomab Stroke Group 2001).

Cytokine targets: Dual, anti-inflammatory and proinflammatory, effects of TNF- α and of IL-6, have been the primary concern in using strategies directed against these *cytokines*. In contrast, IL-1ra directed against IL-1, has found favor with patients with rheumatoid arthritis, and also in cases of acute stroke (Kostulas M 1999, Emsley HC 2008).

Vitamins: Studies have shown that a diet rich in fruits and vegetables has a lower cardiovascular risk, possibly due to antioxidant nutrients: vitamin C, beta-carotene and vitamin E. These might reduce atheroma formation by inhibiting oxidation of LDL (Matsui T 2001). Homocysteine, a sulfur-containing amino acid, is a demethylation product of dietary methionine, which is converted to cysteine by cystathionine B-synthase (a vitamin B6-dependent enzyme), or is remethylated by methionine synthase. The latter reaction is vitamin B12 dependent and requires 5-methyl-tetrahydrofolate, a product of folic acid metabolism that uses methylene-tetrahydrofolate reductase (MTHFR). Defects of cystathionine B-synthase and MTHFR and deficiencies in folic acid, B12, and B6 can lead to raised levels of homocysteine, which have been associated with cardiovascular disease and stroke. Mechanism of homocysteine causing vascular damage include impairment of endothelial functions, endothelial desquamation, oxidation of LDL, increased monocyte adhesion to the vessel wall, impaired vascular response to nitric oxide, and thrombotic tendency mediated by activation of coagulation factors and platelet dysfunction (Matsui T 2001). Studies have shown a correlation between ischemic cerebrovascular disease with MTHFR genotype and serum homocysteine concentration and an interaction with serum folate concentration. Others have also reported association of MTHFR A677V allele with severe carotid stenosis, a moderately elevated homocysteine after methionine loading with increased risk of ischemic stroke in young adults, a graded association of increasing plasma homocysteine with ischemic stroke caused by large-artery atherosclerosis and to a lesser extent small artery disease, an association with risk of silent brain infarction, an association with cervical artery dissection, and microvascular stroke (Sculz JB 1999). However, in contrast cross-sectional and case-control studies, prospective studies generally show less or no predictive ability for plasma homocysteine in coronary disease and stroke.

Predictive Role of Blood Biomarkers in Ischemic Stroke

A recent study reviewed the Medline and EMBASE from 1966 to January 2007 for studies of blood markers in patients with ischemic stroke and an assessment of outcome (death, disability, or handicap). Though cardiac markers showed the most consistent association with poor outcome, many studies were subject to bias. Although some markers had some predictive ability, none of the studies showed any added advantage of biomarkers over a validated clinical model. Thus the clinical usefulness for predicting prognosis in the setting of ischemic stroke is yet to be established, prior to being incorporated into any regimen (Whiteley W 2000).

CONCLUSION

The complex pathophysiology of a stroke encompasses various excitotoxicity mechanisms, inflammatory pathways, oxidative damage and ionic imbalances. Despite significant therapeutic advances in the form of carotid endarterectomy, thrombolytics, anticoagulant therapy, antiplatelet agents, neuroprotective agents, and treating associated risk factors such as hypertension and dyslipidemia have failed to reduce the burden of stroke. Current understanding of inflammation and ischemia has caused a paradigm shift in the perspective of stroke pathogenesis and outcome. It has also opened newer avenues in stroke management and prevention strategies, beyond the realms of antithrombotics. Though one needs to keep abreast with recommended protocols for stroke management, knowledge of the underlying patho-genetic process, aided by laboratory investigations and imaging, may usher in more therapeutic options. Well-designed clinical trials of novel therapeutic agents and strategies will be able to substantiate or refute their clinical usefulness, and confirm the possibility of being incorporated into evidence-based practice guidelines.

Several complex and overlapping pathways underlie the pathophysiology of cell death after ischemic stroke. While pharmaceutical agents can inhibit these pathways at various levels, resulting in effective neuroprotection in experimental models, no single agent intended for neuroprotection has been shown to improve outcome in clinical stroke trials. Refinements in patient selection, brain imaging, and methods of drug delivery, as well as the use of more clinically relevant animal stroke models and use of combination therapies that target the entire neurovascular unit, are warranted to make stroke neuroprotection an achievable goal. Ongoing trials assessing the efficacy of thrombolysis with neuroprotective agents and strategies aimed at extending the therapeutic window for reperfusion therapy promise to enhance the known benefits of reperfusion therapy. Most investigators agree that genomics and proteomics are the most promising recent developments impacting the future of stroke prevention, diagnosis, treatment, and outcome. Although many challenges lie ahead, an attitude of cautious optimism seems justified at this time.

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Chapter 13

INFLAMMATION AND ANTI-INFLAMMATORY AGENTS IN STROKES

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ABSTRACT

Two important pathophysiological mechanisms involved during a stroke are oxidative stress and inflammation. Brain tissue is not well equipped with antioxidant defenses, so reactive oxygen species and other free radicals/oxidants, released by inflammatory cells, threaten the tissue viability in the vicinity of the ischemic core. In a stroke, cerebral blood vessels are the first to be exposed to the ischemic insult, and their reaction to injury sets the stage for the inflammatory response. Molecules released from injured or dying brain cells also contribute to the inflammatory response. Focal ischemia evokes a robust inflammatory response that begins within a few hours of onset and typifies the secondary or delayed response to ischemia. Hence there is a notion that taming post-ischemic inflammation may block secondary events that extend brain injury. However, as noted below, during the later stages in the injury process, inflammation promotes critical events necessary for tissue repair. Here in this chapter, we will discuss the molecular aspects of oxidative stress and inflammation in an ischemic stroke and potential therapeutic strategies that target neuroinflammation and the innate immune system. Currently, little is known about endogenous counter regulatory immune mechanisms. However, recent studies show that regulatory T cells are major cerebral-protective immunomodulators after stroke, suggesting that targeting the endogenous adaptive immune response may offer novel promising neuroprotectant therapies. New research has shown that brain ischemia and trauma elicit strong inflammatory reactions driven by both external and brain cells. The recognition of inflammation as a fundamental

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response to brain ischemia provides novel opportunities for new anti-inflammatory therapies. Hypertension, cardiovascular disease, arterial fibrillation, diabetes mellitus, smoking, and alcohol abuse are all risk factors for a stroke. Clinical observations suggest that inflammation is also a direct risk factor for a stroke. Recent studies have shown that plasma levels of inflammatory cytokines or soluble adhesion molecules are high in non-stroke patients, and anti-inflammatory therapy is effective at reducing the stroke incidence in not only animal models, but in humans as well. Statins have been shown to decrease the stroke incidence via anti-inflammatory effects that are both dependent and independent of their cholesterol-lowering effects. These reports suggest that inflammation might directly affect the onset of a stroke. Here in this chapter, we present the latest findings about the cellular and humoral aspects of immune and inflammatory reactions in the brain. They may have an impact on the treatment of neuro-injuries and ancillary brain diseases, and increase the understanding of the roles infections and immune reactions play in the brain milieu.

INTRODUCTION

Strokes are an important issue in public health due to their high rates of morbidity and mortality, and disability. A stroke is one of the most common causes of death and disability worldwide (Lyod Johnes D 2009). However, current therapeutic strategies for strokes have been largely unsuccessful. One possible explanation is that research and pharmacological management have focused on very early events in brain ischemia. New research has shown that brain ischemia and trauma elicit strong inflammatory reactions driven by both external and brain cells. The recognition of inflammation as a fundamental response to brain ischemia provides novel opportunities for new anti-inflammatory therapies. Hypertension, cardiovascular disease, arterial fibrillation, diabetes mellitus, smoking, and alcohol abuse are all risk factors for a stroke. Clinical observations suggest that inflammation is also a direct risk factor for a stroke. Stroke patients have high levels of inflammatory cytokines in plasma, and immune cells, such as macrophages and T-lymphocytes, are noted within stroke lesions. These inflammatory events are considered as a result of a stroke. However, recent studies show that plasma levels of inflammatory cytokines or soluble adhesion molecules are high in non-stroke patients, and anti-inflammatory therapy is effective at reducing stroke incidence in not only animal models, but in humans as well. Statins have been shown to decrease the stroke incidence via anti-inflammatory effects that are both dependent and independent of their cholesterol-lowering effects. These reports suggest that inflammation might directly affect the onset of a stroke. Microglial cells and blood-derived monocytes/macrophages play important roles in inflammation in both onset and aggravation of stroke lesions. Two important pathophysiological mechanisms involved during an ischemic stroke are oxidative stress and inflammation. Brain tissue is not well equipped with antioxidant defenses, so reactive oxygen species and other free radicals/oxidants, released by inflammatory cells, threaten the tissue viability in the vicinity of the ischemic core. This chapter will discuss the molecular aspects of oxidative stress and inflammation in ischemic strokes and potential therapeutic strategies that target neuroinflammation and the innate immune system. Currently, little is known about endogenous counterregulatory immune mechanisms. However, recent studies show that regulatory T cells are major cerebroprotective immunomodulators after a

stroke suggesting that targeting the endogenous adaptive immune response may offer novel promising neuroprotectant therapies.

Here in this chapter, we present the latest findings about the cellular and humoral aspects of immune and inflammatory reactions in the brain. They may have an impact on the treatment of neuro-injuries and ancillary brain diseases, and increase the understanding of the roles infections and immune reactions play in the brain milieu. Although different mechanisms are involved in the occurrence and development of a stroke, inflammatory response is greatly involved in its sequence (Barone FC 2001). Inflammation is intricately related to the onset of a stroke and to subsequent stroke-related tissue damage. Focal ischemia evokes a robust inflammatory response that begins within a few hours of onset and typifies the secondary or delayed response to ischemia (Figure 3.1). Inflammation within the arterial wall plays a vital role in promoting atherosclerosis (Chomorro A 2004, Elkind MS 2002). Arterial thrombosis (usually associated with ulcerated plaques) is triggered by multiple processes involving endothelial activation, and proinflammatory and prothrombotic interactions between the vessel walls and circulating blood elements. An elevated stroke risk has been linked to high levels of serologic markers of inflammation such as C-reactive protein (Ridker PM 2000), ESR, interleukin-6, TNF-alpha, and soluble intercellular adhesion molecule (sICAM) (Tanne D 2000). These events are promoted in part by the binding of cell adhesion molecules from the selectin and immunoglobulin gene families expressed on endothelial cells to the glycoprotein receptors expressed on the neutrophil surface. As evidence, reduced ischemic infarction is observed in ICAM-1 knockout mice and infarction volumes are increased in mice that overexpress P-selectin (Connolly ES 1996 and 1997). The proinflammatory molecule P-selectin is expressed on vascular endothelial within 90 min after cerebral ischemia, ICAM-1 by 4 h, and E-selectin by 24 h (del Zoppo G 2000).

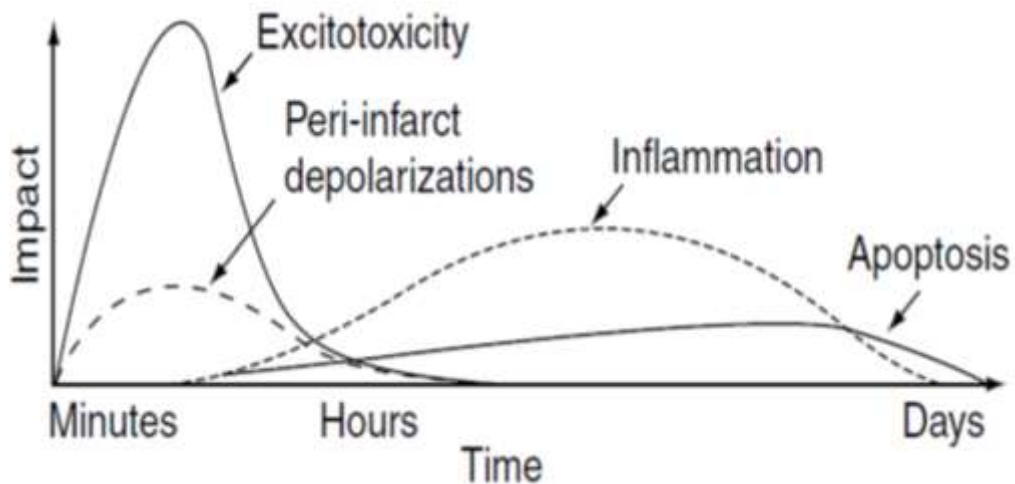


Figure 3.1. Inflammation Putative cascade of damaging events in focal cerebral ischemia. Very early after the onset of the focal perfusion deficit, excitotoxic mechanisms can damage neurons and glia lethally. In addition, excitotoxicity triggers a number of events that can further contribute to the demise of the tissue. Such events include peri-infarct depolarizations and the more-delayed mechanisms of inflammation and programmed cell death. The x-axis reflects the evolution of the cascade over time, while the y-axis aims to illustrate the impact of each element of the cascade on final outcome (courtesy of Dirnagl et al. 1999).

Inhibiting both selectin adhesion molecules and activation of complement reduces brain injury and suppresses neutrophil and platelet accumulation after focal ischemia in mice (Huang J 1999). In humans, neutrophil and complement activation significantly worsened outcomes in a clinical trial using humanized mouse antibodies directed against ICAM Enlimomab (Enlimomab 2000). Hence, the complexities of interactions between multiple pathways will have to be carefully considered for optimal translation to the clinic. Ischemic stroke-related brain injury itself triggers inflammatory cascades within the parenchyma that further amplify tissue damage (del Zoppo G 2000). As reactive microglia, macrophages, and leukocytes are recruited into ischemic brain, inflammatory mediators are generated by these cells as well as by neurons and astrocytes. Inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin-1 (IL-1), and monocyte chemoattractant protein-1 (MCP-1) are key inflammatory mediators, as evidenced by attenuated ischemic injury in mutant mice with targeted disruption of these genes (Barone FC 1999, Huggs PM 2002, Ledecolla C 2001, Boutin H 2001).

Initially after occlusion, there is a transient upregulation of immediate early genes encoding transcription factors (e.g., c-fos, c-Jun) that occurs within minutes. This is followed by a second wave of heat shock genes (e.g., HSP70, HSP72) that increases within 1–2 h and then decreases by 1–2 days. Approximately 12–24 h after a stroke, a third wave comprised of chemokines and cytokines is expressed (e.g., IL-1, IL-6, IL-8, TNF-alpha, MCP-1, etc.). Whether or not these three waves are causally related is not known. Nevertheless, therapies that seek to target these pathways need to be carefully timed to match the complex temporal evolution of tissue injury. Inflammatory Cascades stimulate both detrimental and potentially beneficial pathways after ischemia. For example, administering TNF-alpha neutralizing antibodies reduces brain injury after focal ischemia in rats (Nawashiro H 1997), whereas ischemic injury increases in TNF receptor knockout mice (Bruce AJ 1996). In part, these contrasting results may reflect signal transduction cascades activated by TNF-R1 and TNF-R2, with TNF-R1 augmenting cell death and TNF-R2 mediating neuroprotection (Fountaine V 2002). Similarly, the peptide vascular endothelial growth factor (VEGF) exacerbates edema in the acute phase of cerebral ischemia, but promotes vascular remodeling during stroke recovery (Zhang ZG 2000). Ultimately, the net effect of these mediators depends upon the stage of tissue injury or the predominance of a single signaling cascade among multiple divergent pathways.

In the early stage of cerebral ischemia, circulating leukocytes including neutrophils, macrophages, and lymphocytes migrate and adhere to the injured endothelial cells and infiltrate into the ischemic brain region *via* a dysfunctional blood–brain barrier (Lakhan SE 2009). The inflammatory response is also characterized by endogenous microglia activation following focal cerebral ischemia (Hellenbeck JM 1996, Morioka T 1993, Wang Q 2007). The infiltration of blood derived leukocytes and macrophages liberate toxic and inflammatory mediators involved in a “no reflow phenomenon” and amplify the ischemic brain injury (Yilmaz G 2010).

Post-ischemic reflow, either due to collateral circulation establishment or therapeutic recanalization, leads to the generation of reactive oxygen species (ROS), which then stimulates ischemic cells, including neurons and astrocytes to secrete inflammatory cytokines, chemokines and adhesion molecules (Ceulemans AG 2010). Once the inflammatory cascade is activated, inflammatory cells can release a variety of cytotoxic agents, including more cytokines, matrix metalloproteinases (MMPs), nitric oxide (NO) and induce more cell

damage as well as disruption of the blood–brain barrier (BBB) and extracellular matrix (Denton GH 2003, Emsley HC 2002). Blocking various aspects of the inflammatory cascade was shown to attenuate ischemic brain injury from an experimental stroke (Han HS 2003) although this has yet to be demonstrated clinically (Becker K 2001). Exploration of ischemia-induced inflammatory related biomarkers therefore is important and necessary to understand the mechanism of ischemia-induced inflammatory brain injury.

During cerebral ischemia, inflammatory cytokines interleukin (IL)-1 β , IL-6 and tumor necrosis factor (TNF), are extremely up-regulated (up to 40- to 60-fold) at least in the brain within the first 24 h of an experimental stroke model (Clausen 2005 and 2008, Hill JK 1999, Lambertsen 2005). These three cytokines are able to affect the volume of the ischemic induced tissue damage in a rodent experimental stroke model (Yang GY 1999). These cytokines also increase in cerebro-spinal fluid (CSF) and circulating blood after an ischemic stroke in humans (Allan SM 2005, McCoy MK 2008 and Whiteley W 2009). Although IL-1 β , IL-6, and TNF α are among the most investigated cytokines in CSF and blood in human stroke patients, their availability and mechanism of action in the human brain in the early phase after an experimental stroke remain unknown (Ginsberg MD 2008). Besides these three cytokines, other factors such as IL-8, IL-10, CD40L, IFN γ , IL-1 α , IL-17, and TGF β also participate in the inflammatory response after an ischemic stroke (Pang L 2001 and Smith CJ 2004). Understanding the cellular production of cytokines in normal brain, and the time profile and the cellular sources of these cytokines, and the possibilities of their transport in the ischemic territory in the early phase after the onset of a stroke is crucial to unveiling the mechanisms by which these cytokines potentially affect brain damage after an ischemic stroke. A panel of cytokines has been shown to vary their presence in the peripheral blood after cerebral ischemia, which is associated with changes of their levels in the brain tissue or the cerebral spinal fluid (CSF). However, the principal shortcoming including a high individual variance of circulating cytokine levels limited the establishment of clinical criteria for control groups. Using a modified method (mosaic ELISA kits), Zeng et al. has detected a cluster of cytokines in each sample at the same time. This novel method required less volume of a blood sample and significantly decreased biological error compared to other studies using single ELISA assay.

To establish a relevant control group is critical in clinical stroke studies. In most cases, studies are limited to the choice of healthy volunteer as a control group. Chronic disease such as diabetes, hypertension, hyperlipidemia also influence a chronic inflammatory response and result in the release of numerous circulating cytokines (Spraner J 2003 Greenberg AS 2006 and Wisse BE 2004). Stroke patients usually suffer a significantly higher frequency of cardiovascular risk factors compared to the healthy controls. Hence, the difference in circulating cytokine levels between the stroke group and the control group could not exclude the influence of cardiovascular risk factors. To eliminate this factor, Zeng et al. chose age, sex, cardiovascular risk factors matched normal controls to eliminate the influence of cardiovascular risk factors. As a result, they found that among the most studied panel of pro-inflammatory cytokines, IL-6 was the only one that was not up-regulated in plasma after cerebral ischemia but also correlated with the severity of neurological impairment.

Numerous studies have revealed a close relationship between pro-inflammatory cytokine release and post-stroke, inflammatory injury (Dnes A 2010, Lambertsen KL 2005 and Spalata G 2006). Although many studies focus on the clinical significance of individual cytokine, each source of circulating cytokines is unclear. A study on the source of cytokines in

peripheral blood is useful to better understand the function of cytokine both in peripheral and brain inflammation. There are three major hypotheses:

- (1) Cytokines are released into circulating blood by inflammatory cells in ischemic brain tissue *via* an impaired blood–brain barrier;
- (2) Cytokines are secreted and released by activating leukocytes such as neutrophils, lymphocytes and monocytes;
- (3) Cytokines are released by peripheral immune organs

However, some researchers (Planas Am 2006, Wang Q 2007, Tutolomonodo A 2008) did not find a positive relationship between plasma cytokine protein level and their RNA level in leukocyte. Therefore, the source of circulating cytokines needs to be further studied in the future. The interaction of cytokines is another important issue which is largely unknown. It is interesting that Zeng et al. adopted Bayesian network (BN) learning procedure in an attempt to understand the interactions (inter-influencing network) among the 8 cytokines in protein and mRNA level after an ischemic stroke. The BN method is used widely in many fields, including protein–protein interactions (Bradford JR 2006, Hack CE 2010), gene function analysis (Zhao B 2011), and brain functional connectivity using MRI (Burge J 2009). Zeng et al. used this method to explore the interaction among circulating pro-inflammatory cytokines in patients with acute ischemic stroke. Although the data need to be further confirmed by others, the original result revealed a suspected complex circulating pro-inflammatory cytokine network model with positive and negative feedback. Pro-inflammatory cytokine up-regulation in plasma and compensatory immunity, depression in leukocyte involve in peripheral inflammatory response to ischemic brain injury. IL-6 activation is a potential therapeutic target for stroke therapy to attenuate secondary inflammatory injury. The detailed cytokine network during ischemia development calls for further investigation with animal experiments *in vivo* and *in vitro*.

The most common cause of a stroke is the sudden occlusion of a blood vessel by a thrombus or embolism, resulting in an almost immediate loss of oxygen and glucose to the cerebral tissue. Although different mechanisms are involved in the pathogenesis of a stroke, increasing evidence shows that ischemic injury and inflammation account for its pathogenic progression (Muir KW 2007). Cerebral ischemia triggers the pathological pathways of the ischemic cascade and ultimately causes irreversible neuronal injury in the ischemic core within minutes of the onset (Dirnagl U 1999). However, a much larger volume of brain tissue surrounding this ischemic core, known as the penumbra, can be salvaged if cerebral blood flow is promptly restored. Thus, the original definition of the ischemic penumbra referred to areas of the brain that were damaged but not yet dead, offering the promise that if proper therapies could be found, one could rescue brain tissue after a stroke and reduce post-stroke disability. Despite advances in the understanding of the pathophysiology of cerebral ischemia, therapeutic options for acute ischemic stroke remain very limited (Donnan GA 2008). Only one drug is approved for clinical use for the thrombolytic treatment of acute ischemic stroke in the US and that is intravenous recombinant tissue plasminogen activator (rt-PA). When delivered within three hours after symptom onset, rt-PA reduces neurological deficits and improves the functional outcome of stroke patients. However, this improvement in recovery is achieved at the expense of an increased incidence in symptomatic intracranial hemorrhage, which occurs in ~6% of patients. Furthermore, since the large majority of patients with acute

ischemic stroke do not go to the hospital within three hours of stroke onset most do not receive rt-PA treatment (Furlan AJ 2003). Consequently, the successful treatment of acute ischemic stroke remains one of the major challenges in clinical medicine. This chapter will provide a brief overview of the current understanding of the inflammatory mechanisms involved in an acute ischemic stroke and the neuroprotective agents that can curtail neuroinflammation and potentially show utility in the treatment of a stroke. Neuroprotective treatments or therapies block the cellular, biochemical, and metabolic elaboration of injury during exposure to ischemia. Of the more than 100 neuroprotective agents that reached randomized clinical trials in focal ischemic stroke, none has proven unequivocally efficacious, despite success seen in preceding animal studies (O'Collins VE 2006). However, the field trials of the past have greatly increased our understanding of the fundamental biology of ischemic brain injury and have laid a strong foundation for future advance. New anti-inflammatory targets continue to be identified, which is an important area of translational medicine in acute stroke. Overall, the prospects for safe neuroprotective therapies to improve stroke outcomes remain promising (De la Ossa NP 2007).

IMMUNE ACTIVATION AND SYSTEMIC INFLAMMATION BEFORE A STROKE

The immune system plays a role in the development of atheromatous plaque and in plaque rupture (Koenig W 2007). The production of atheromatous plaque involves local inflammation within the wall of blood vessels (Croce K 2007, Hansson GK 2006). The cells in the plaque include macrophages, CD4 and CD8 T cells (Xu QB 1990, Jhonsson L 1990). One target of the immune attack in atherosclerosis is oxidized low density lipoprotein (oxLDL), which is highly immunogenic (Kobayashi K 2005). Some of the T cells in plaque are likely to be specific for oxLDL, as tolerance to oxLDL reduces the severity of atheroma through a mechanism involving regulatory T cells (Treg cells) (Mor A 2007, Van Puijvelde Gh 2006). The entry of leukocytes into tissue is mediated by endothelial expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin and L-selectin (Moller F 2005, Galkina E 2006). Chemokines are members of a large superfamily of small molecules that have a role in the movement of leukocytes through tissues. This occurs through interaction with chemokine receptors on cells (Colobran R 2007). Chemokines play a role in the entry of lymphocytes into atheromatous plaque. For example, CXCR6 is required for the entry of CXCR6 cells into atheromatous plaque (Galkina E 2007) and CXCL10 attracts effector T cells into plaque (Heller EA 2006). Another factor that regulates atherogenesis is an expression of CD40, which is a glycoprotein expressed on many cells including endothelial cells. Ligation of CD40 on endothelium results in endothelial activation. CD40 interacts with CD40 ligand (CD40L), which is expressed on CD41 T cells. CD40 is important in the entry of T cells into plaque and also in plaque activation (Mach F 1997) T cells that have entered an atheromatous plaque may release cytokines that lead to plaque rupture (Tedgui A 2006).

Systemic inflammation, measured as high levels of C-reactive protein (CRP) (Tanne D 2006) or fibrinogen (Zhu YC 2006), is a risk factor for a stroke (Elkind MS 2006). There is debate as to whether, in individual patients, there is a causal relationship between elevated

levels of these proteins and stroke incidence. A recent study suggests that patients with echolucent plaques, that are likely to rupture, have higher levels of systemic inflammation than patients with plaques that are less likely to rupture (Withnoto AT 2006). Thus, inflammatory processes may predispose to stroke. Furthermore, in experimental animals, systemic inflammation induced by injection of lipopolysaccharide (LPS) causes exacerbation of damage through pathways involving interleukin-1 (IL-1) (McColl BW 2007). The brain is considered to be a site of relative immunological privilege, because of the relative lack of rejection of foreign tissue that has been transplanted into the brain (Simpson E 2006). Microglia, which are the resident antigen-presenting cells in the brain, are thought to provide constant surveillance of tissue injury (Nimerjhan A 2005) and become activated when injury occurs. However, for an adaptive immune response to occur, antigen-presenting cells must travel to draining lymph nodes. Microglia are thought to have little or no ability to travel to draining lymph nodes, which has been put forward as one reason for the relative immune privilege of the brain (Melchior B 2006). Nevertheless, there is immune surveillance of the brain and there are local immune responses to brain injury (Lucas SM 2006). As well as parenchymal microglia, the brain also has perivascular macrophages (Kim WK 2006) that are hematogenous in origin. Furthermore, in strokes, and in other pathological states, breakdown of the blood-brain barrier (Wang PY 1993) means that the injured tissue becomes relatively more exposed to immune processes with entry of blood-borne inflammatory cells. Macrophages of the brain are able to travel to draining lymph nodes to initiate adaptive immune responses (Fabreik BO 2005).

POST-ISCHEMIC INFLAMMATION

Although for many years the CNS was considered an immune-privileged organ, it is now well accepted that the immune and the nervous system are engaged in bidirectional crosstalk. Moreover, mounting data suggest that in the brain, as in peripheral organs, inflammatory cells participate in tissue remodeling after injury. Microglial cells are the resident macrophages of the brain and play a critical role as resident immunocompetent and phagocytic cells in the CNS. Ekdahl and colleagues (Ekdahl CT 2009) reported an increased number of activated microglial cells up to 16 weeks after two hours MCAO in rats. After activation by ischemia, microglia can transform into phagocytes and they can release a variety of substances, many of which are cytotoxic and/or cytoprotective. Microglia may exert neuroprotection by producing neurotrophic molecules such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor I (IGF-I), and several other growth factors. There is substantial evidence that activated microglial cells in response to ischemia have the potential of releasing several pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, as well as other potential cytotoxic molecules including NO, ROS, and prostanoids (Lucas SM 2006). Astrocytes, like microglia, are capable of secreting inflammatory factors such as cytokines, chemokines, and NO (Swanson RA 2004). Cytokines upregulate the expression of cell adhesion molecules (CAMs). Within four to six hours after ischemia onset, circulating leukocytes adhere to the vessel walls and migrate into the brain with subsequent release of additional pro-inflammatory mediators and secondary injury in the penumbra. Neutrophils are the earliest

leukocyte subtype to show substantial upregulation in gene expression studies and to infiltrate areas of brain ischemia (see figure 3.2).

Recently, Shichita et al. (Shichita T 2009) demonstrated an infiltration of γ δT cells 3 days after the onset of ischemia in a mouse model, along with a production of IL-17 which amplifies the inflammatory cascade. IL-23 from infiltrating macrophages appears to produce IL-23 which attracts the infiltrating γ δT cells. Blocking a specific γ δT cell receptor with an antibody effectively reduced three day infarct volumes, even when treatment was initiated at 24 hours after onset of cerebral ischemia. Targeting these γ δT cells may offer a clinical opportunity with a longer therapeutic window to prevent the secondary inflammatory expansion of cerebral damage after a stroke. The described post-ischemic neuroinflammatory changes lead to dysfunction of the brain tissue. Therefore, therapeutic targeting of the neuroinflammatory pathways in acute ischemic strokes has become an important area of research in translational medicine.

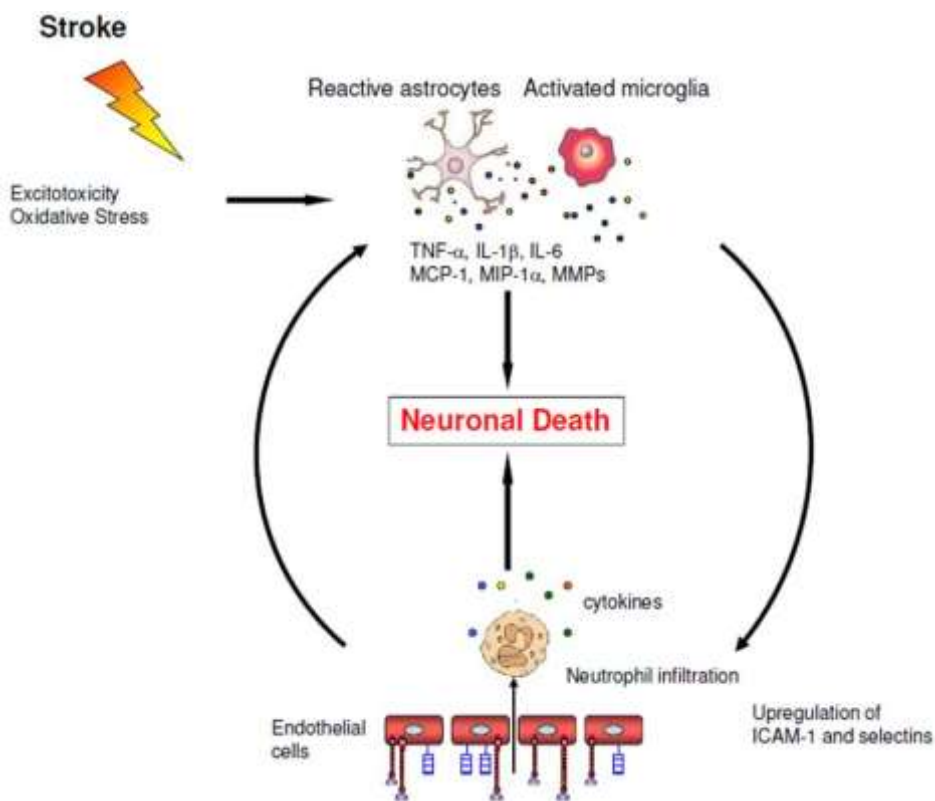


Figure 3.2. Postischemic inflammatory responses. Excitotoxicity and oxidative stress caused by the initial ischemic event activate microglia and astrocytes, which react by secreting cytokines, chemokines and matrix metalloproteases (MMP). These inflammatory mediators lead to an upregulation of cell adhesion molecules on endothelial cells, allowing blood derived inflammatory cells, mainly neutrophils, to infiltrate the ischemic brain area. Neutrophils themselves also secrete cytokines which cause a further activation of glial cells. These processes all result in neuronal cell death and enhance the damage to the ischemic brain.

Cytokines and Brain Inflammation

Cytokines are a group of small glycoproteins that are produced in response to an antigen and were originally described as mediators for regulating the innate and adaptive immune systems. Cytokines are thus upregulated in the brain in a variety of diseases, including strokes. In the brain, cytokines are expressed not only in the cells of the immune system, but are also produced by resident brain cells, including neurons and glia (Barone FC 1999). In addition, it has been shown that peripherally derived cytokines are involved in brain inflammation. Thus, peripherally derived mononuclear phagocytes, T lymphocytes, NK cells and polymorphonuclear leukocytes produce and secrete cytokines and might contribute to inflammation of the CNS (Ferrarese C 1999).

The most studied cytokines related to inflammation in an acute ischemic stroke are tumor necrosis factor- α (TNF- α), the interleukins (IL), IL-1 β , IL-6, IL-20, IL-10 and transforming growth factor (TGF)- β . While IL-1 β and TNF- α , appear to exacerbate cerebral injury, TGF- β and IL-10 may be neuroprotective (Zhu Y 2002, Spera PA 1998). Increased production of proinflammatory cytokines and lower levels of the anti-inflammatory IL-10 are related to larger infarctions and poorer clinical outcome. Elevated IL-1 β mRNA expression occurs within the first 15-30 min after permanent MCAO and elevated IL-1 β protein expression occurs a few hours later and remains elevated for up to 4 days (Caso JR 2007). There are studies that correlate an increase in the levels of IL-1 β after ischemia with worsening of the infarct severity. For example, Yamasaki et al. (Yamasaki Y 1995) demonstrated that intraventricular injection of recombinant IL-1 β after MCAO increases the formation of brain edema, the volume of the size and the influx of neutrophils. In addition, IL-1 β deficient mice presented smaller infarcts in comparison with wild-type mice (Boutin H 2001). High circulating IL-1 β elevates circulating IL-6, other well-known cytokine that is upregulated following cerebral ischemia (Clark WM 1999). Moreover, the serum level of IL-6 correlates with brain infarct volume (Acalovschi D 2003) and is a powerful predictor of early neurological deterioration (Vila N 2001). On the other hand, Clark et al. (Clark WM 2001) demonstrated that infarct size and neurological function were not different in animals deficient in IL-6 after transient CNS ischemia. This suggests that IL-6 does not have a direct influence on acute ischemic injury. IL-20 is induced when IL-1 β modulates p38 MAPK and the NF- κ B pathway. IL-20 in turn induces the production of IL-6. Inhibition of IL-20 by a specific mAb significantly ameliorated the brain ischemic infarction in rats following MCAO (Chen WY 2009). Several approaches are under investigation for managing IL-1 in a stroke (Table 1). IL-1 acts via membrane receptors (IL-1R), which can be blocked by a receptor antagonist (IL-1RA). In a randomized trial for an acute stroke, IL-1RA readily crossed the blood-brain barrier, was safe to use, and seemed to afford some benefit, particularly for patients with cortical infarcts (Gueorguieva I 2008). IL-10 is an anti-inflammatory cytokine that acts by inhibiting IL-1 and TNF- α , and by suppressing cytokine receptor expression and receptor activation as well. As a consequence, IL-10 could provide neuroprotection in acute ischemic strokes. Both central and systemic administration of IL-10 to rats subjected to MCAO significantly reduced infarct size 30 min to three hours post MCAO (30). In acute ischemic strokes, elevated concentrations of IL-10 in CSF have been found (Tarkowski E 1997). Moreover, patients with low plasma levels (<6 pg/ml) of IL-10 during the first hours after a stroke were three times more likely to have worsening neurological symptoms within

48 hours following the stroke (Vila N 2001). IL-10 also seems to mediate the reduction in infarct size by regulatory T cells.

Table 1. Clinical studies of agents targeting inflammatory pathways in acute ischemic strokes

S. No	Neuro-protective agent	Mode of action	Reference
1	Enlimomab	Anti-ICAM-1 monoclonal antibody	Enlimomab 2001
2	Cerovive (NXY-059)	Nitrone-based free radical trapping agent	Lyden PD et al. 2007 Shuyaib A et. al. 2007
3	Recombinant human IL-1RA	Interleukin-1 receptor antagonist	Emsley HC et al. 2005
4	UK-279, 276	Neutrophil inhibitory factor	Krams M et al. 2003
5	Tirilazad	Lipid peroxidation inhibitor	Bath PM et al. 2001
6	Ginsenoside	Ca ²⁺ channel antagonist	Liu X et al. 2009
7	Edaravone MCI-186	Free radical scavenger	Edaravone Study Group 2003
8	Acetaminophen (Paracetamol)	Anti-pyretic effect	Van Breda EJ et al. 2005
9	Minocycline	Anti-inflammatory	Lampl Y et. al. 2007
10	ONO-2506 (Arundic Acid)	Astrocyte modulator	Pettigrew LC et al. 2006

Adapted from Shah et al., 2009.

Chemokines and Brain Inflammation

Chemokines, for example, monocyte chemoattractant protein 1, are a class of cytokines that guide the migration of blood borne inflammatory cells, such as neutrophils and macrophages, towards the source of the chemokine. Consequently, they play important roles in cellular communication and inflammatory cell recruitment. Expression of chemokines such as MCP-1, macrophage inflammatory protein-1 α (MIP-1 α), and fractalkine following focal ischemia is thought to have a deleterious effect by increasing leukocyte infiltration (Kim JS 1995). The level of a variety of chemokines has been found to increase in animal models of ischemia and their inhibition or deficiency has been associated with reduced injury (Soriano S 2002, Huges PM 2002). Mice without the chemokine receptor CCR2 are protected against ischemia-reperfusion injury (Dimitrijevic OB 2007).

Cellular Adhesion Molecules

There is increasing evidence that cellular adhesion molecules (CAMs) play an important role in the pathophysiology of an acute ischemic stroke. CAMs are upregulated in the first days after a stroke by various cytokines and are responsible for the adhesion and migration of the leukocytes. Leukocytes roll on the endothelial surface and then adhere to the endothelial cells. The interaction between leukocytes and the vascular endothelium is mediated by three main groups of CAMs: the selectins, the immunoglobulin gene superfamily, and the integrins. Selectins, especially E- and P-selectins are upregulated and mediate leukocyte rolling and

recruitment during the early stages of ischemia (Zhang, R 1998) Among the immunoglobulin family member, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 have been the most extensively investigated in cerebral ischemia. Within hours after the onset of stroke, ICAM-1 expression increases upon stimulation by cytokines (Lindsberg PJ 1996). Patients with acute ischemic strokes had higher soluble intracellular adhesion molecule-1 (sICAM-1) levels compared to patients without cardiovascular disease. Moreover, sICAM-1 levels were significantly higher in patients who died compared to those who survived (Rallidis LS, 2009). High sICAM-1 levels on admission are associated with early death in ischemic middle-aged stroke patients, suggesting a pathogenic role of inflammation in the evolution of ischemic strokes.

A number of animal studies have shown that after transient and permanent focal ischemia the upregulation of CAMs, especially ICAM-1, P- and E-selectin, preceded the invasion of neutrophils into the brain. There is ample evidence from animal models of MCAO that expression of CAMs is associated with cerebral infarct size. Thus, genetic ablation of CAMs resulted in reduced infarct size, which could be mimicked by treatment with anti-CAM antibodies (Zhang RL 1994, Gossev AV 1998). Inhibition of leukocyte activation and infiltration into the ischemic cerebral tissue has, therefore, been an important area of neuroprotection research. Thus far, anti-CAM treatment has not been successful in patients with acute ischemic strokes. However, further translational research into the therapeutic targeting of CAM is ongoing. The spatiotemporal profile of CAMs is still largely unresolved, even though they are crucial for efficient anti-inflammatory therapies. More knowledge of the spatiotemporal profile of CAMs may lead the way to successful application and monitoring of promising anti-inflammatory treatment strategies after a stroke.

Matrix Metalloproteinases

MMPs are a family of proteolytic enzymes that are responsible for remodeling the extracellular matrix and that can degrade all its constituents. Expression of MMPs in the adult brain is very low to undetectable, but many MMPs are upregulated in the brain in response to injury (Montaner J 2001).

Neurons, astrocytes, microglia, and endothelial cells have all been shown to express MMPs after injury. A stroke is associated with a biphasic disruption of the blood brain barrier (BBB) leading to vasogenic edema and hemorrhage and experimental studies have shown that that BBB breakdown and hemorrhage results from the expression and activation of MMPs (Asaie M 2001). MMP-2 and MMP-9 have been implicated in cerebral ischemia. Elevated MMP-9 levels were found in brain tissue and in serum from patients with acute ischemic strokes and in animal models of strokes beginning at 12 h after permanent MCAO (Park KP 2009).

MMP-9 is normally absent and this is the major MMP associated with neuroinflammation. Early (day 1) MMP-9 inhibition reduced infarction of day 14. However, the benefit was lost when the treatment was delayed until day 3 and the stroke pathology was exacerbated when the administration was delayed until day 7. These studies all suggest that MMP inhibition could have a beneficial effect on the outcome of a stroke, but the effect will depend on the timing of treatment in relation to the stage of brain injury (Zhao BQ 2006).

PROINFLAMMATORY CYTOKINES IN STROKES

A stroke is the third leading cause of death and a major cause of disability in industrialized countries. An ischemic stroke is the most common type of stroke, occurring in approximately 80% of all strokes. A less common type of stroke is a hemorrhagic stroke, which occurs due to a subarachnoid hemorrhage and/or an intracerebral hemorrhage. Hypertension, cardiovascular disease, arterial fibrillation, diabetes mellitus, obesity, smoking, and alcohol abuse are risk factors for a stroke (Goldstein LB 2011), even if there are slight differences in the influence of these factors between ischemic stroke and hemorrhagic stroke. However, some stroke patients do not have any of these risk factors, suggesting that other risk factors exist. For many years, clinical observations showed that plasma levels of inflammatory cytokines were increased after stroke onset, and immune cells, especially monocytes/macrophages and T-lymphocytes, existed in stroke lesions and related to exaggerate brain damage. In the clinical setting, elevated plasma levels of inflammatory cytokines, C-reactive protein (CRP), and chemokines are associated with future cardiovascular risk (Blake GJ 2002). Plasma levels of soluble intercellular adhesion molecule-1 (sICAM-1) and sE-selectin were observed to be increased both in large intracranial artery disease and small-artery disease (Fassbender K 1999), and plasma levels of ICAM-1 and monocyte chemoattractant protein-1 (MCP-1) were noted to be high in patients with ischemic stroke and myocardial infarction (Sánchez-Moreno C 2004, Arakelyan A 2005). Epidemiological studies have shown that elevated leukocyte count was associated with the risk for first-time myocardial infarction and ischemic stroke (Prentice RL 1982, Fawa M 1987, Gillum RF 1994) and the risk of recurrent myocardial infarction and ischemic stroke in a high-risk population (Grau AJ 2004). These observations indicate that inflammatory events occur in stroke patients and increase the risk of stroke recurrence. Recently, both clinical and animal studies revealed that these inflammatory events occurred prior to stroke onset. Plasma levels of soluble vascular cell adhesion molecule-1 (sVCAM-1), sICAM-1, sE-selectin, and MCP-1 were elevated in patients with essential hypertension in the absence of other diseases (Blann AD 1994, Madej A 2005, Desouza CA 1997). Anti-inflammatory strategies were shown to suppress the incidence of a stroke in both human and animal models. These reports suggest that inflammation might be a risk factor for a stroke. Atherosclerosis is one of the major risk factors for a stroke, and monocytes/macrophages affect the brain indirectly by inducing unstable plaques and plaque rupture in atherosclerotic lesions (Newby AC 2008). It is well recognized that atherosclerosis is an inflammatory disease and macrophages play important roles in the initiation and the progression of atherosclerotic lesion. Accumulation of monocytes/macrophages in the vascular wall occurs early during atherosclerosis (Ross R 1999). In addition to phagocytosis of oxidized low-density lipoproteins, macrophages secrete interleukin- 1β (IL- 1β), tumor necrosis factor- α (TNF- α), and transforming growth factor- β 1 (TGF- β 1). These inflammatory cytokines and growth factors induce endothelial dysfunction, smooth muscle cell migration and proliferation, and extracellular matrix production as fibrous plaques. During later disease stages, activated macrophages secrete several classes of neutral extracellular proteases, including serine proteases, cathepsins, and matrix metalloproteinases (MMPs) (Dollery AM 2006). Blood monocytes have already expressed low levels of a few MMPs (Bar-Or A 2003); however, contact with matrix leads to rapid upregulation of a broad spectrum of MMPs. Cell biology experiments identify mechanisms by which excessive MMP

production can cause plaque rupture, either directly by destruction of extracellular matrix or indirectly through actions that promote death of macrophages (Johnson JL 2008) and vascular smooth muscle cells (Newbay AC 2006). Rupture of unstable plaques may lead to thrombotic stroke onset. Inflammatory cytokines, such as IL-1 β , IL-6, and TNF- α , are secreted by activated microglial cells and macrophages in stroke lesions and induce the expression of chemokines, which recruit more circulating monocytes/macrophages into lesions and lead to further brain damage. However, the role of each cytokine in a stroke is complicated.

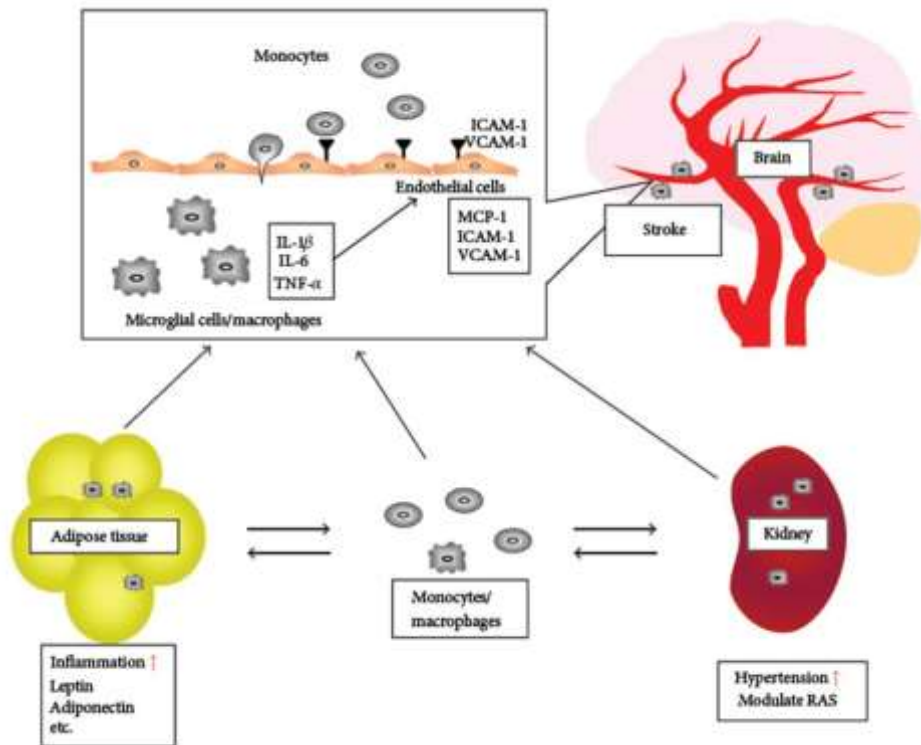


Figure 3.3. Inflammation: Monocytes/macrophages modulate adipose tissue and kidney functions and accelerate the onset of a stroke. Monocytes/macrophage infiltration into adipose tissue stimulates secretion of leptin and inflammatory cytokines and suppresses secretion of adiponectin, which induce systemic inflammation, endothelial cell dysfunction, and monocytes/macrophage activation. Monocytes/macrophages infiltration into kidney modulates renin-angiotensin system and increase blood pressure, which also induces endothelial cell dysfunction and monocytes/macrophages activation. Endothelial cells express MCP-1 and adhesion molecules, which induce monocytes chemotaxis, adhesion, and migration into subendothelial lesions. Microglial cells and infiltrated monocytes/macrophages in brain induce cerebrovascular damages and cause stroke onset.

Interleukin-1 β

Recently, IL-1 β has been considered a therapeutic target for a stroke. Chronic increases in IL-1 β expression in the brain led to leukocyte infiltration and increased MCP-1 and ICAM-1 expressions in a mouse model, which is a phenotype also seen in stroke lesions. In addition, a number of studies have demonstrated that inhibiting the release or action of IL-1 markedly

reduced ischemic cerebral damage in animal models. IL-1 α and IL-1 β double knockout mice exhibited dramatically reduced ischemic infarct volume compared with wild-type mice (Boutin H 2001). In a meta-analysis of animal model studies, IL-1 receptor antagonist (IL-1Ra), which represents the most advanced approach to modify IL-1 actions, reduced infarct volume in models of focal cerebral ischemia (Banwell V 2009). In humans, a phase II clinical trial of intravenous IL-1Ra compared with the placebo in patients with acute strokes is currently underway (Emsley HCA 2005). Further, IL-1Ra gene polymorphism represents a risk factor for an ischemic stroke (Olsson S 2012). These reports suggest that inhibition of IL-1 β signals can prevent the onset of a stroke.

Interleukin-6

A prospective cohort study and systemic review revealed that plasma levels of IL-6 were associated with poor outcome after both ischemic and hemorrhagic strokes (Whiteley C 2009); however, it was not clear whether IL-6 increased before or after stroke onset. Animal models showed less association between IL-6 and strokes. IL-6 could not induce adhesion molecules and MCP-1 mRNA expressions in cerebrovascular endothelial cells derived from SHRSP. Mice deficient in IL-6 showed similar stroke lesion volume and neurological function as control mice in an acute ischemic injury model (Clark WM 2000). Furthermore, IL-6 mediates anti-inflammatory effects in addition to its proinflammatory role (Scheller A 2011). Therefore, its manipulation can have either detrimental or beneficial effects. Further studies are required to clarify the role of IL-6 in a stroke.

Tumor Necrosis Factor Alpha (TNF- α)

Increased serum and cerebro-spinal fluid levels of TNF- α have been found in patients 24 hours, 1 week, and 2 weeks after a stroke, and these increases correlate with infarct volume and severity of neurological impairment (Zaremba J 2001). However, previous reports suggest that TNF- α has a dual role in brain injury (Hallenbeck JM 2002). Blockade of TNF- α actions reduced infarct volume after permanent middle cerebral artery occlusion in BALB/C mice with the dimeric type I soluble TNF receptor, which binds to TNF- α and antagonizes its action (Nawashiro H 1997a). In contrast, TNF- α pretreatment was neuroprotective against permanent middle cerebral artery occlusion in BALB/C mice with reduction of infarct size, macrophages, and CD11b-positive neutrophils (Nawashiro H 1997b). In addition to these observations, pentoxifylline, an anti-inflammatory agent, attenuated damage of a stroke via the dual role of TNF- α . Pentoxifylline treatment increased serum levels of TNF- α , but not IL-1 β and IL-6, and dose dependently prevented the occurrence of spontaneous brain damage by reducing macrophage infiltration into the lesion in SHRSP (Ban C 2004), suggesting a protective role of TNF- α . On the other hand, pentoxifylline reduced brain edema in a rat model of transient focal cerebral ischemia through a decline in TNF- α production (Vakili A 2011), suggesting a deleterious role of TNF- α . Based on crucial evidence that implicate TNF α as a mediator of acute brain damage, a number of pharmacological strategies have been followed by different investigators to either inhibit the synthesis or to neutralize the action of

TNF α and to assess the effect of such treatments on the outcome of ischemia and trauma. The pharmacological approaches that have been applied to improve the outcome after brain injury were based on: (a) inhibition of TNF α synthesis of low molecular weight molecules; and (b) neutralization of the biological effect of TNF α by proteins such as specific antibodies or binding proteins. Although anti-TNF- α strategies have proved beneficial in other clinical settings such as inflammatory bowel disease, there are no clinical trials of anti-TNF- α agents in strokes. Further studies are therefore required to clarify the role of TNF- α in strokes.

MCP-1

CC chemokine ligand (CCL2) is known as MCP-1 and is a potent mononuclear cell attractant. MCP-1 is synthesized by several cell types, such as monocytes/macrophages, T-lymphocytes, smooth muscle cells, endothelial cells, and even cerebrovascular endothelial cells. Expression of MCP-1 is upregulated by inflammatory cytokines. Serum levels of MCP-1 are high in patients with ischemic strokes and myocardial infarction (Arkeyalan A 2005), which might be interpreted as a stroke-induced increases in inflammatory events. It is reported that plasma levels of MCP-1 were elevated in patients with essential hypertension in the absence of other diseases (Madej A 2005). The MCP-1-deficient mice model is a unique model to elucidate the role of macrophages in a stroke (Huges PM 2002). Compared with control mice, infarct volume was smaller in MCP-1-deficient mice 24 hours after middle cerebral artery occlusion, and a reduction of phagocytic macrophage accumulation within infarcts and the infarct border in MCP-1 deficient mice 2 weeks after middle cerebral artery occlusion. In addition, MCP-1 deficient mice produced less IL-1 β in ischemic tissue. This means that MCP-1 and IL-1 β are key factors of macrophages in stroke lesions.

Adipokines

Obesity is also recognized as a risk factor for a stroke, because obesity is associated with hypertension and inflammation via secretion of adipokines, such as adiponectin, leptin, Resistin, adipisin, plasminogen activator inhibitor-1, and inflammatory cytokines (Mathieu P 2009, Yiannikouris A 2010). It is well known that macrophage infiltration into adipose tissue induces inflammation in adipose tissue and influences these adipokine secretions (Bouloumié A 2005). The most commonly studied adipocytokines are leptin and adiponectin. There are a lot of reports about the association of leptin and adiponectin with strokes, and leptin and adiponectin show differential association patterns with ischemic strokes (Kim BJ 2012). It is reported that higher leptin levels and lower adiponectin levels were found in stroke patients (Gerdes S 2012). On the other hand, there is controversial reports that adiponectin, but not leptin, levels are recognized as a predictor of the risk for a stroke (Pruger C 2012), or that leptin, but not adiponectin; levels are recognized as a predictor of the risk for strokes in men, but not women (Söderberg S 2009). It is not clear whether adiponectin and leptin are useful predictors of strokes in obese subjects; however, adiponectin and leptin might directly influence stroke incidence. It is reported that leptin stimulates macrophages and that adiponectin suppresses it. Leptin increases the mRNA and protein levels of IL-1 β , IL-6, IL-

12, TNF- α , cyclooxygenase-2, and MCP-1 in macrophages and endothelial cells (Loffreda S 1998). Adiponectin inhibits pro-inflammatory signaling in human macrophages (Folko EJ 2009) and promotes macrophage polarization toward an anti-inflammatory phenotype (Ohashi K 2010). Adiponectin also increases IL-10, an anti-inflammatory cytokine, as well as mRNA expression in human monocyte-derived macrophages (Kumada M 2004). In addition, both adiponectin and leptin receptors are expressed in the brain, suggesting that these adipokines might be directly associated with a stroke (Thundiyil J 2012).

ANTI-INFLAMMATORY STRATEGIES

There are several reports that treatment with drugs that have anti-inflammatory properties can prevent strokes not only in animal models, but also in humans.

Statins

Rosuvastatin treatment significantly delayed the onset of a stroke and attenuated the transcription of inflammatory biomarkers (Sironi L 2005). Clinical studies using statins already use inflammatory events as endpoints for stroke prevention. In healthy persons without hyperlipidemia but with elevated high-sensitivity CRP levels, rosuvastatin, which lowered high-sensitivity CRP as well as cholesterol levels, reduced the incidence of strokes and myocardial infarction by 50% relative to placebo (Ridker PM 2008). A meta-analysis of statin trials showed that statins might reduce the incidence of all strokes by decreasing LDL-cholesterol without increasing the incidence of hemorrhagic strokes (Amarenco P 2004). In addition to cholesterol dependent effects, cholesterol-independent effects of statins on strokes have also been recognized (Vaughan CJ 2003). However, statin treatment increases the risk of hemorrhagic strokes in patients with a history of cerebrovascular disease, even though it also clearly decreased the risk of an ischemic stroke (Vergouwen MD 2008). Therefore, patients undergoing statin treatment should be carefully monitored to avoid achieving very low levels of cholesterol, which are known risks for hemorrhagic strokes (Puddey IB 1996).

Thiazolidinediones

Thiazolidinediones, including rosiglitazone and pioglitazone, are peroxisome proliferator activated receptor- $\gamma\gamma$ (PPAR- γ) agonists used in the treatment of type 2 diabetes. A systemic review showed that 6 Mediators of Inflammation rosiglitazone and pioglitazone were similarly effective in reducing infarct volume and protecting neurologic function in a rodent model of focal or global cerebral ischemia (White AT 2010). Pioglitazone delayed the onset of a stroke by improving vascular endothelial dysfunction and brain inflammation in SHRSP. Pioglitazone suppressed macrophage infiltration, MCP-1 and TNF- α gene expressions in the brain (Nakamura T 2007). Rosiglitazone induced upregulation of CD36 in macrophages and enhanced the ability of microglia to phagocytose red blood cells, which helped to improve hematoma resolution, and improved functional deficits in an intracerebral hemorrhage mouse

model (Xhao H 2009). In humans, the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROACTIVE) (Dormandy JA 2005) showed that pioglitazone significantly reduced the risk of a recurrent stroke in high-risk patients with type 2 diabetes (Wilcox R 2007). On the other hand, one report showed that compared with pioglitazone, rosiglitazone was associated with an increased risk of a stroke, heart failure, and all-cause mortality and an increased risk of the composite of acute myocardial infarction, strokes, heart failure, or all-cause mortality in patients of 65 years or older (Graham DJ 2010).

Other Inflammatory Drugs

Low-dose acetylsalicylic acid (aspirin) also delayed the onset of a stroke in SHRSP via suppression of inflammation. Aspirin reduced salt induced macrophage accumulation and MMP-9 activity at the stroke-negative area in the cerebral cortex of SHRSP (Ishizuka T 2008). Frequent aspirin use might also confer a protective effect for the risk of strokes in humans (Tymianski M 2011). Terutroban, a specific thromboxane/prostaglandin endoperoxide receptor antagonist, decreased cerebral mRNA expressions of IL-1 β , transforming growth factor- β , and MCP-1 and increased survival in SHRSP. These effects were similar to rosuvastatin and aspirin (Gelosa P 2010). The Prevention of cerebrovascular and cardiovascular Events of ischemic origin with terutroban in patients with a history of ischemic strokes or transient ischemic attack (PERFORM) study was started in February 2006 (Bousser MG 2009). Recently, it was reported that PERFORM study did not meet the predefined criteria for non-inferiority, but showed similar rates to terutroban and aspirin for the primary endpoint, such as a composite of fatal or nonfatal ischemic strokes, fatal or nonfatal myocardial infarction, or other vascular death (Bousser MG 2011). These reports indicate that antiplatelets agents, which also have anti-inflammatory properties, could suppress inflammation and prevent the onset of a stroke.

Inflammation and Ischemic Tolerance

Inflammatory and immune responses play important roles following an ischemic stroke. Inflammatory responses contribute to damage and also contribute to repair. Injury to tissue triggers an immune response. This is initiated through activation of the innate immune system. There is microglial activation in a stroke. This is followed by an influx of lymphocytes and macrophages into the brain, triggered by the production of proinflammatory cytokines. This inflammatory response contributes to further tissue injury. There is also a systemic immune response to a stroke, and there is a degree of immunosuppression that may contribute to the stroke patient's risk of infection. This immunosuppressive response may also be protective, with regulatory lymphocytes producing cytokines and growth factors that are neuroprotective. The specific targets of the immune response after a stroke are not known, and the details of the immune and inflammatory responses are only partly understood. The role of inflammation and immune responses after a stroke is twofold. The immune system may contribute to the damage after a stroke, but may also contribute to repair processes. The possibility that some of the immune responses after a stroke may be neuroprotective is

exciting and suggests that the deliberate enhancement of these responses may be a therapeutic option.

The suppression of inflammation appears to be the means by which ischemic preconditioning protects against a stroke. It is known that a sublethal ischemic event confers protection against subsequent lethal ischemia, in various organs, including the brain (Dhodha VK 2004). In experimental studies, animals that have been subjected to preconditioning have reduced inflammation after a subsequent ischemic challenge and reduced expression of inflammatory molecules (Bowen KK 2006). These changes are thought to be associated with changes in expression of genes such as hypoxia inducible factor 1 (HIF1) (Bernaudin M 2002, Dhodha VK 2004). In summary, it appears that immune suppression after a preconditioning ischemic episode prevents the acute harmful immune response after a subsequent ischemic episode. There is evidence that inflammation participates in tissue damage in strokes, and this is brought about by the activation of the innate immune system. Immunosuppression after a stroke has occurred may be too late to reduce this damage, although further work is required. After the acute injury of a stroke, there appears to be a naturally occurring state of immunosuppression, during which infection can occur. This immunosuppression appears to be due, in part, to circulating T cells. There is evidence that the immune system, possibly through such T cells, but also by other types of T cells, may be able to assist in neural repair. There is some animal work that suggests that tolerization to brain antigens improves outcomes after a stroke. Thus, immune and inflammatory responses after a stroke are both good and bad. Therapeutic possibilities based on this data include the early use of antibiotics to prevent infection (currently undergoing clinical trial), inhibition of the early inflammatory/immune response, although it is perhaps likely, that doing so after the stroke has occurred may be too late, and perhaps enhancing protective immune responses to speed neural repair.

CONCLUSION

In conclusion, in the presented work, we sought to provide a brief overview of the current understanding of inflammatory mechanisms involved during an acute ischemic stroke and neuroprotective agents that can curtail neuroinflammation and could have utility in the treatment of a stroke (see Table 1). As discussed, evidence suggests that post-ischemic oxidative stress and inflammation contribute to brain injury and to the expansion of the ischemic lesion. On the other hand, an adequate adaptive immune response after acute brain ischemia also plays an important role in response to ischemic injury as shown by the tremendous potential of Treg cells to prevent secondary infarct growth by counteracting the production of proinflammatory cytokines and by modulating the activation of lymphocytes and microglia in the ischemic brain (Liesz A 2009). These results provide new insights into the immunopathogenesis of acute ischemic strokes and could lead to new approaches that involve immune modulation using Treg cells. To date, 1,026 drugs have been tested in various animal models, of which 114 underwent clinical evaluation (De la Ossa NP 2007). The greater part of the agents studied until now have failed. Consequently, rt-PA remains the only agent shown to improve stroke outcomes in clinical trials, despite the many clinical trials conducted. However, its use is limited by its short therapeutic window (three hours), by its

complications derived essentially from the risk of hemorrhage, and by the potential damage by R/I injury. Because of these drawbacks the optimum treatment of cerebral focal ischemia remains one of the major challenges in clinical medicine.

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Chapter 14

MAJOR ADVANCES IN THE TREATMENT OF STROKES

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ABSTRACT

The translation of neuroprotective agents for ischemic stroke from bench-to-bedside has largely failed to produce improved treatments since the development of tissue plasminogen activator (tPA). One possible reason for lack of translation is the failure to acknowledge the greatest risk factor for stroke, age, and other common comorbidities such as hypertension, obesity, and diabetes that are associated with stroke. In this chapter, we highlight both mechanisms of studying these factors and results of those that have been addressed.

We also discuss the potential role of other lifestyle factors associated with an increased stroke risk, such as sleep fragmentation and/or deprivation. Furthermore, many proposed therapeutic agents have targeted molecular mechanisms occurring soon after the onset of ischemia despite data indicating delayed patient presentation following ischemic stroke.

Modulating inflammation has been identified as a promising therapeutic avenue consistent with preliminary success of ongoing clinical trials for anti-inflammatory compounds such as minocycline. In this chapter, we review the role of inflammation in stroke and in particular, the role of inflammatory cell recruitment and macrophage phenotype in the inflammatory process. Emerging evidence indicates an increasing role of neuro-immune crosstalk, which has led to increased interest in identification of peripheral biomarkers indicative of neural injury. It is our hope that identification and investigation of factors influencing stroke pathophysiology may lead to improved therapeutics.

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Keywords: Neuroprotective, Plasminogen activator, hypertension, Ischemic, inflammatory, therapeutics, pathophysiology, clinical trials, biomarkers

INTRODUCTION

Acute ischemic stroke remains a condition of high morbidity and mortality. Until now, the only established therapy has been intravenous (IV) tissue-type plasminogen activator (tPA). Only 3–10% of patients with acute ischemic stroke receive this treatment. On the basis of data from part 3 of the European Collaborative Acute Stroke Study (ECASS III), the time window for beneficial treatment of ischemic stroke with IV tPA has been extended from 3 to 4.5 h after the onset of stroke symptoms. Beyond that window of opportunity, and additionally to IV treatment, interventional stroke therapy has assumed an important role for the treatment of acute ischemic stroke. Currently, new promising pharmacological and mechanical treatment options are being established as routine procedures to achieve a further improved outcome for stroke patients.

The main challenge in stroke therapy is how to rescue patients who have survived the ischemic insult, considering that by the time they are evaluated, the majority might have passed the 3-h window in which to receive t-PA therapy. Therefore, the prime issues are: i) how to extend the window of treatment; ii) how to minimize the effect of reperfusion injury, considering that the damaging effect due to reperfusion sometimes could be greater than that due to ischemia itself; iii) how to reduce the risks associated with t-PA therapy beyond 3 h; and iv) how to regenerate infarcted brain areas to regain neurological function. New advances that combine the basic understanding of pathobiology of the disease and translational research, including drug delivery approaches and surgical interventions could address the complex issues associated with stroke therapy.

Stroke is a leading cause of death, long-term disability, and socioeconomic costs, highlighting the urgent need for more effective treatments. Intravenous administration of tissue plasminogen activator (t-PA) is the only FDA-approved therapy to re-establish cerebral blood flow. However, because of increased risk of hemorrhage beyond 3 h post stroke, few stroke patients (1–2%) benefit from t-PA; t-PA, which has neurotoxic effects, can also aggravate the extent of reperfusion injury by increasing blood-brain barrier permeability. An alternative strategy is needed to extend the window of intervention, minimize damage from reperfusion injury, and promote brain repair leading to neurological recovery. Reactive oxygen species (ROS), generated soon after ischemia and during reperfusion and thereafter, are considered the main mediators of ischemic injury. Antioxidant enzymes such as catalase, superoxide dismutase, etc. can neutralize ROS-mediated injury but their effective delivery to the brain remains a challenge. In this chapter, we review various therapeutic approaches including surgical interventions, and discuss the potential of nanoparticle-mediated delivery of antioxidants for stroke therapy.

Stroke is a sudden loss of brain function resulting from interference with the blood supply to the central nervous system (CNS). Normal cerebral blood flow (CBF) is approximately 50–60 ml/100g/min. The reduction in CBF below 20 ml/100g/min results in an electrical silence and less than 10 ml/100g/min causes irreversible neuronal injury (Hakim M. 1998, Jones T. H. 1981). Lack of blood circulation to the brain deprives neurons of necessary glucose and

oxygen. Neurons are the impulse transmitters; hence they require constant supply of energy. Up to 85% of all strokes are of ischemic origin, with most attributable to blockage of one or more cerebral artery by blood clots, with resulting reduction in cerebral perfusion. The remaining stroke cases are hemorrhagic, involving either intracerebral or subarachnoid hemorrhage.

MECHANISMS OF NEURONAL INJURY AND THE THERAPEUTIC POSSIBILITIES

Cerebral ischemia results in a number of hemodynamic, biochemical, and neurophysiological alterations (Ostrowski R. P. 2006). A series of complex acute, subacute and chronic events occur after the incidence of stroke and reperfusion (Jean W. C. 1998). Ischemic injury involves energy failure, loss of cell ion homeostasis, acidosis, increased intracellular calcium excitotoxicity, free radical-mediated toxicity, and pathological permeability of the blood-brain barrier (BBB). Free radicals, specifically reactive oxygen species (ROS) that are generated soon after ischemia, as well as in later stages of ischemic reperfusion (e.g., by inflammatory cells), are the fundamental mediators of reperfusion injury.

The development of hypoxic-ischemic neuronal injury is significantly influenced by the release of excitatory neurotransmitters, primarily glutamate and aspartate in the brain. This process is called excitotoxicity and is activated by depletion of cellular energy stores. Glutamate, normally stored inside the synaptic terminals, is released in the extracellular space in a depleted energy state, which then results in the opening of calcium channels associated with N-methyl- D aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxanole propionate (AMPA) receptors. This leads to an influx of calcium, sodium and chloride ions and efflux of potassium ions. (Siesjo B. K. 1989, Hademenos G. J. 1997, Garcia J. H. 1994). The influx of calcium is responsible for the activation of a series of destructive enzymes such as proteases, endonucleases and lipases that allow release of cytokines and other inflammatory mediators, resulting in the loss of cellular integrity (Siejo B. K. 1981, Becker K. J. 1998). Inflammatory mechanisms play an important role in tissue injury by inducing the rapid production of many different inflammatory mediators. Within 30 minutes after ischemia and reperfusion, leukocytes recruited to the ischemic area activate mediators of inflammation such as oxygen free radicals, cytokines, and nitric acid.

A vast amount of data implicates oxygen-derived free radicals (especially superoxide and hydroxyl radicals) and high-energy oxidants (such as peroxynitrite) as mediators of inflammation in ischemia/reperfusion injury (Cuzzocrea S. 2001). Free radicals are highly reactive molecules generated predominantly during cellular respiration and normal metabolism. Imbalance between cellular production of free radicals and the ability of cells to defend against them is referred to as oxidative stress (Valko M. 2007).

After brain injury by ischemic stroke, the production of ROS dramatically increases, leading to tissue damage via several different cellular, molecular pathways, and mechanical pressure (Figure 4.1).

ROS can cause damage to cellular components such as lipids, protein, and nucleic acids, leading to subsequent cell death (Janardhan V. 2004). Damage can become more widespread due to weakened cellular antioxidant defense systems in ischemia.

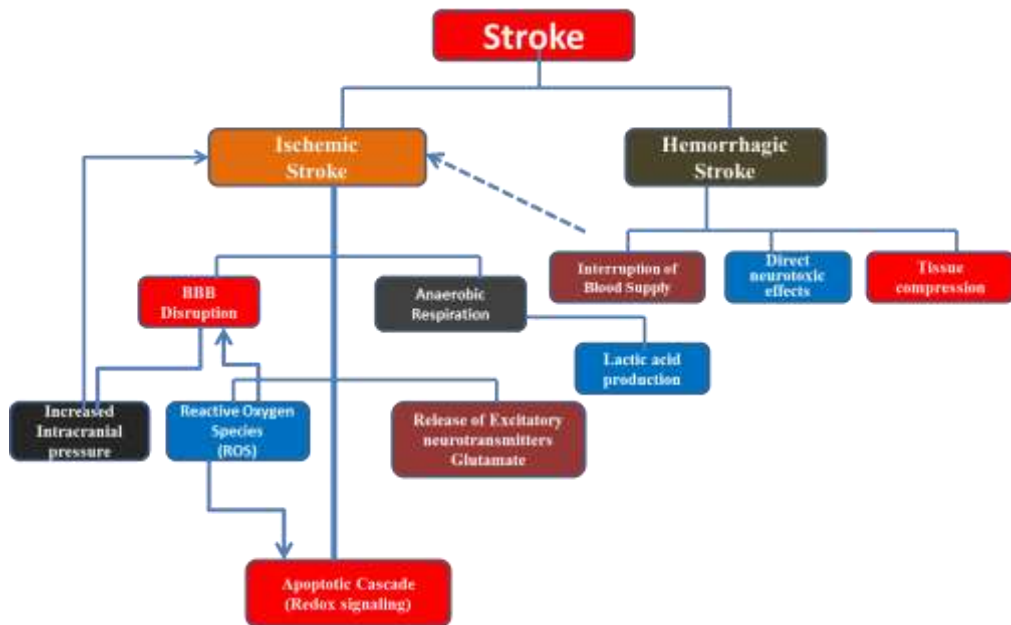


Figure 4.1. Schematic showing different types of stroke and cascade of events following ischemia.

ROS are the major stimulators of inflammatory cytokine production (such as interleukin-1, tumor necrosis factor- α , and interferon- γ) and protease secretion by microglia, leukocytes, and resident cells of the neurovascular unit (Haddad J. J. 2002). As these neuroinflammatory mechanisms become activated, alterations in cytokine profiles, adhesion-molecule expression, and tight-junction components mediate further vascular leakage. Significant evidence exists suggesting that ROS are involved in every fundamental physiological step that leads to neuronal death (Cao W. 1988) and thus are considered an important target in developing an effective stroke therapy (Juurlink B. H. 1997).

Following the onset of ischemic stroke, cerebral blood flow is disrupted throughout the affected region of the brain. Blood flow disruption to the tissue is not uniform due to the presence of collateral circulation resulting in a flow gradient. Consequently, a “core” region of infarct develops in which flow is severely reduced, often approximating upwards of a 90% decrement, and tissue undergoes necrosis within minutes as insufficient adenosine triphosphate (ATP) is present to maintain homeostatic ionic gradients and metabolic functions (Astrup J. 1977). Surrounding this core region in which blood flow is severely reduced is the “penumbra”, a tissue distribution in which flow is less severely reduced and remains typically around 35% of baseline flow (Astrup J. 1977). Characterized by normal cellular membrane potential but a disruption in the ability for normal action potential firing, tissue initially residing within the penumbral region progresses to cellular death, ultimately resulting in an expanded core lesion without restoration of cerebral blood flow (Lo E. H. 2008). As such, therapeutic development has focused on achieving reperfusion via tissue plasminogen activator (tPA) and/or restoring homeostasis via administration of ‘neuroprotective’ pharmacologic agents intended to disrupt the ischemic injury cascade.

Current research in stroke has focused on developing neuroprotective therapies that target many mechanisms involved in stroke pathophysiology.

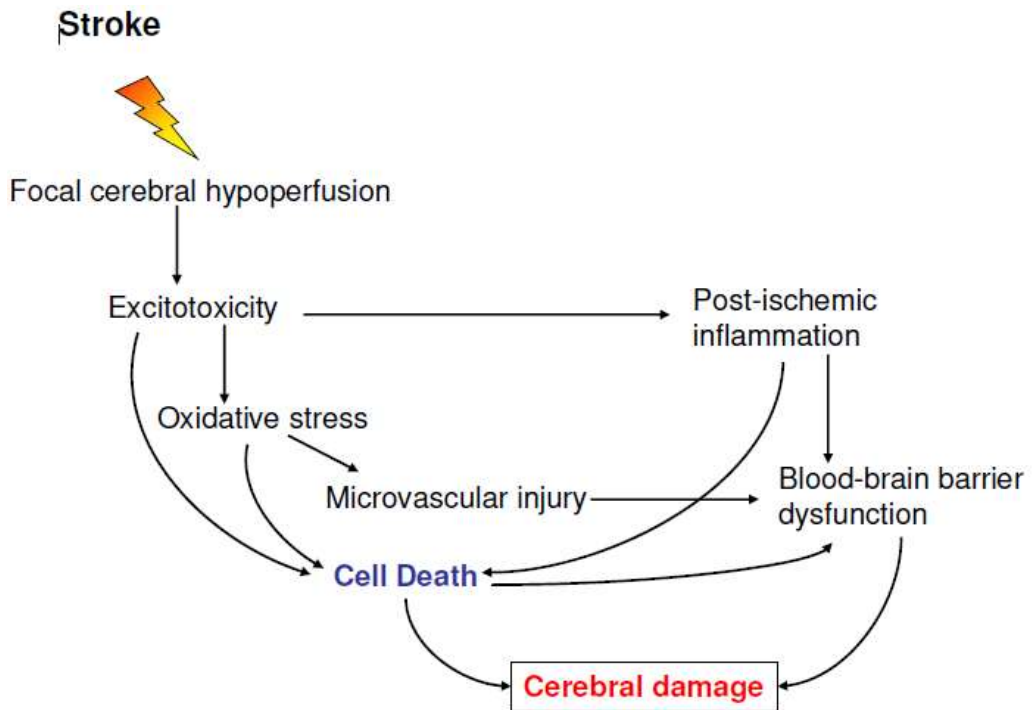


Figure 4.2. Ischemic cascade leading to cerebral damage. Ischemic stroke leads to hypoperfusion of a brain area that initiates a complex series of events. Excitotoxicity, oxidative stress, microvascular injury, blood-brain barrier dysfunction and postischemic inflammation lead ultimately to cell death of neurons, glia and endothelial cells. The degree and duration of ischemia determines the extent of cerebral damage.

Studies from animal and cell models have greatly expanded our knowledge in this field and form the basis of clinical trials of neuroprotective therapies. Results have been variable; it is very likely that no single therapy will be satisfactory, and that multiple agents working through different mechanisms or novel agents that target more than disease mechanism may offer the best hope for a future neuroprotective therapy. In this chapter, we address several disease mechanisms of pathogenesis in stroke, along with some examples of therapies targeting each of these mechanisms.

CURRENT STATUS OF NEUROPROTECTANT DEVELOPMENT: DRUG DEVELOPMENT SHORTCOMINGS

Investigation of neuroprotection and the associated concepts have gone through various periods of apparent focus as described by Lo previously (Lo E. H. 2008). Early studies, initiated by Astrup and colleagues, focused on understanding the effect of ischemia produced across a range of cerebral blood flow measurements on neurophysiology and electrical conduction (Astrup J. 2008).

The second phase of investigation sought to identify molecular mechanisms of injury with an emphasis on the acute period of injury. Following molecular biology studies was the

discovery and implementation of numerous imaging modalities in both preclinical and clinical studies for assessing both changes in blood flow and the evolution of both the penumbra and infarct core (Lo E. H. 2008). While these various developments have provided much insight and utility in both preclinical and clinical studies, successful translation of a neuroprotectant from bench-to-bedside has yet to occur despite identification of over 1000 “successful” neuroprotectants in preclinical studies and initiation of over 100 clinical trials (O’Collins V. E. 2006).

The reasons for lack of translation and failure are presently unknown but clear discrepancies exist between preclinical models and the clinical population most commonly afflicted by ischemic stroke. These include the age and general health of the population being studied as well as the time point of treatment initiation. Similarly, the endpoints frequently used in preclinical and clinical studies are often different. Furthermore, the vast majority of preclinical work has been conducted in rodents, species that are more distant phylogenetically from the human population in comparison to nonhuman primates. Nonhuman primates more closely resemble humans in vascular anatomy, experience striatal damage following ischemic stroke more comparably to the human, and are gyrencephalic in nature like humans (Fukuda S. 2003). Consequently, nonhuman primates represent perhaps the most ideal preclinical model, but are frequently not utilized due to cost, lack of availability, and expertise required for care and use. For these reasons, nonhuman primate models are not discussed further. The following sections elaborate on these concepts in an attempt to convey the contribution of common comorbidities seen in the clinical population to stroke risk and outcome. Furthermore, the potential impact of other lifestyle factors such as sleep fragmentation and/or deprivation, often related to an altered sleep-wake cycle by something such as shift work, is discussed.

In treating acute ischemic stroke, two primary strategies are followed: 1) limiting the ischemic insult by early reperfusion and 2) interfering with the pathobiochemical cascade leading to ischemic neuronal damage.

In developing an effective therapy, one must also weigh the deleterious consequences of reperfusion injury versus the effects of delay in resuming blood supply to the brain (Wagner K. R. 2004).

Within an hour of ischemia, two layers of cerebrovascular tissue develop: the inner core displaying necrosis of both neuronal as well as supporting glial elements, and the outer layer of less severe ischemia called the ischemic penumbra. The cells within the ischemic penumbra can be recovered if timely therapeutic intervention is administered. The size of the ischemic penumbra (portion of the brain that can be rescued) diminishes with time, as the infarct core progressively becomes hypoperfused tissue that cannot be rescued following reperfusion. Ischemic damage in the penumbra, however, is reversible and is the target of rescue via re-establishing blood flow (Fisher M. 2004).

DRUGS USED IN ACUTE STROKE THERAPY

Drug therapy is a relatively recent approach to the treatment of stroke, and a large amount of research is focused on finding effective new drugs that can minimize stroke damage. The most relevant drugs evaluated for acute stroke therapy are summarized in Table 4.1.

Table 4.1. Drugs used in endovascular therapy for acute stroke

Group	Drug	Effect
Drugs to improve blood flow	Heparin Abciximab Ancrod	Antithrombotic Antiplatelet Fibrinogen depleting
Thrombolytics	Streptokinase Urokinase Prourokinase Alteplase Retepase Tenecteplase Tissue plasminogen activator Alfimeprase BB10153 Desmoteplase	Plasminogen activators Direct acting fibrinolytics
New drugs under evaluation	V10153 Microplasmin	

PLASMINOGEN ACTIVATORS

Plasminogen activators are the primary drugs used as thrombolytic agents in acute stroke therapy. These drugs act by converting plasminogen into plasmin. Plasmin unlocks fibrinogen, fibrin monomers and cross-linked fibrin (as found in a thrombus) into fibrin degradation products (Lisboa R. C. 2002).

Streptokinase a clot buster bind to plasminogen and the streptokinase/plasminogen complex then serves as a plasminogen activator. It is a protein derived from group CS-hemolytic streptococci, which showed significant rates of ICH and systemic hemorrhage (Cornu C. 2000); thus, it is no longer used for acute stroke therapy; rather, Urokinase, a serine protease with a plasma half-life of 14 min, is used (Lisboa R. C. 2002). The randomized trial of intra-arterial infusion of urokinase within 6 h of middle cerebral artery stroke: the middle cerebral artery embolism local fibrinolytic intervention trial (MELT), suggested that intra-arterial fibrinolysis has the potential to increase the likelihood of excellent functional outcome (Ogawa A. 2007). Prourokinase (r-prourokinase) is the proenzyme precursor of urokinase. Despite the favorable results in the PROACT (Prolyse in Acute Cerebral Thromboembolism) I and II trials (de Zoppo D. J. 1998, Furlan A. 1999), the FDA did not approve its use in IA stroke therapy.

Alteplase (recombinant human tissue-type plasminogen activator; rtPA) is a serine protease with a plasma half-life of 3.5 min. This short half-life and the limited penetration of the clot, because of strong binding with surface fibrin, delay recanalization and increase the risk of recurrent occlusion. By the administration of heparin or antiplatelet drugs, reocclusion may be reduced.

Moreover, rtPA seems to have some neurotoxic properties, including activation of metalloproteinases, which may result in increased blood–brain barrier permeability leading to

cerebral hemorrhage and edema, as well as amplification of calcium currents through the N-methyl-D-aspartate receptor. This leads to excitotoxicity and neuronal death (Kaur J. 2004). Nevertheless, rtPA represents the first line therapy in the treatment of acute ischemic stroke. More recently licensed plasminogen activators are variants of tPA. These include Reteplase, a truncated tPA variant with a longer half-life and Tenecteplase, a bioengineered tPA variant. Because of their longer half-lives, Reteplase and Tenecteplase can be given as a bolus injection, thereby simplifying administration (Llevadot J. 2001). A case study reported 84% recanalization and no ICH with IA Reteplase in combination with mechanical thrombolysis (Qureshi A. 2002). A pilot clinical trial with Tenecteplase, was terminated owing to symptomatic and asymptomatic ICHs (15% and 23%, respectively) (Haley E. C. 2005). Direct fibrinolytics and new fibrinolytic agents currently under development were built upon advances of tPA derivatives developed to produce less bleeding (Mellot M. J. 1995).

Alfimeprase (ARCA Biopharma Broomfield, CO, USA), a recombinant, truncated form of fibrolyase, has been developed to accelerate lysis. It is designed to dissolve clots by directly degrading fibrin when delivered through a catheter directly into the thrombus and is inactivated locally by circulating alpha-2 macroglobulin. Because there is no need for plasmin generation, alfimeprase has the potential to degrade fibrin more rapidly than tPA. This should result in faster recanalization and lower hemorrhagic risk (Deitcher S. R. 2005). Nevertheless, alfimeprase failed to meet the key efficacy end points of revascularization in the phase II trial CARNEROS-1 (Proof-of-Concept Study of the Safety and Efficacy of Alfimeprase to Rapidly Open Arteries and Restore Brain Function Following a Stroke). The full results of this trial have not yet been published.

BB10153 (British Biotech, Oxford, England), a variant form of plasminogen, has the plasminogen activator cleavage site replaced with a thrombin cleavage site. Like plasminogen it binds to fibrin and gets converted to plasmin by fibrin-bound thrombin. In a phase II dose-escalation study 34% of patients achieved complete lysis in the infarct-related artery. There were no intracranial bleeds (Gibson C. M. 2006) and based on these data, BB10153 is undergoing continued investigation. Desmoteplase (Paion, Aachen, Germany), a recombinant analog of the full-length plasminogen activator isolated from the saliva of the vampire bat, demonstrated safety in the Desmoteplase in Acute Ischemic Stroke (DIAS), Dose Escalation of Desmoteplase for Acute Ischemic Stroke (DEDAS), and DIAS-II trials (Furlan A. J. 2006). A new trial (DIAS-III) was stopped because of lack of efficacy in the desmoteplase-treated patients. The thrust of this study was to determine whether the window for fibrinolytic therapy could be extended beyond 3 h (Hacke W. 2005).

The success of plasminogen activators is, at least in part, highly dependent on the amount of plasminogen in the thrombus. Newer drugs do not depend on the availability of plasminogen.

Ancrod (Viprinex; Neurobiological Technologies, Emeryville, CA, USA) is a defibrinogenating agent derived from the venom of the Malayan pit viper. By defibrinogenating blood an anticoagulant effect is promoted. To study the safety and efficacy of ancrod in patients with acute ischemic stroke administered within 6 h of stroke onset, the Ancrod Stroke Study was performed. No significant difference in overall mean scores on the Scandinavian Stroke Scale could be demonstrated. Those patients with ancrod-induced 6-h fibrinogen levels of 130 mg/dL or less had a marginally significantly better neurological outcome on the Scandinavian Stroke Scale, mortality, and Barthel Index than ancrod-treated patients with higher fibrinogen levels (The Ancrod Stroke investigators, 1994).

V10153 (Vernalis, Winnersh, UK) is a novel modified recombinant variant of human plasminogen, which is activated to plasmin by thrombin. Thrombin, as the key enzyme involved in blood clot formation, is utilised to initiate clot destruction (Davson K. M. 1994). V10153 selectively induces lysis of newly formed clots, which should result in a reduced risk of hemorrhage. The V10153 Acute Stroke Thrombolysis Trial (VASTT) was a phase II dose-escalation multicenter study. The trial has been halted because of significant hemorrhagic complications. The trial enrolment is closed and data are currently being analyzed.

Microplasmin (ThromboGenics, Heverlee, Belgium) is a truncated form of plasmin that is more resistant to antiplasmin. In a rabbit stroke model, IV microplasmin infusion resulted in a high rate of clot lysis without increasing the rate of ICH (Lapchak P. A. 2002). These findings were confirmed in the MITI-IV (Microplasmin in Treatment of Ischemic Stroke-IntraVenous) trial, a phase II multicenter, randomized, double-blinded, placebo-controlled ascending-dose clinical trial. Data are yet to be published.

ADJUVANT DRUGS

Because fibrinolytic agents also have prothrombotic properties, early reocclusion has been demonstrated in 34% of the patients treated with IV rtPA (Alexandrov A. V. 2002). Therefore, the adjuvant use of antithrombotic agents is common practice. IV heparin for systemic anticoagulation during the periprocedural phase of endovascular stroke therapy is controversial. The potential advantages, including augmentation of the thrombolytic effect, prevention of acute reocclusion and reduction in the risk of catheter-related embolism, have to be weighed against the potentially increased risk of ICH when heparin is combined with a thrombolytic agent (delzeppo G. J. 1998). The use of glycoprotein (GP) IIb/IIIa antagonists, such as ReoPro (abciximab – an intravenous platelet aggregation inhibitor, is a monoclonal antibody directed against the platelet glycoprotein GP IIb–IIIa receptor), Integrilin (eptifibatide), or Aggrastat (tirofiban) in ischemic stroke remains investigational. In pilot studies, no major ICH was seen in combination with standard rtPA therapy (Pancioli M. 2008). Conversely, the AbESTT (Abciximab in Emergent Stroke Treatment) Trial-II, a phase III study, evaluating the safety and efficacy of abciximab in acute ischemic stroke, including patients who awoke with stroke symptoms, was permanently stopped owing to a high rate of intracranial hemorrhage in the abciximab-treated patients (Adams H. P. 2008). The data for the use of GP IIb/IIIa inhibitors in conjunction with intraarterial thrombolysis are limited to case reports. The number of cases with symptomatic ICH varied between 0 and 23% (Nogueira R. J. 2008)

ENDOVASCULAR THERAPY OF ACUTE STROKE

The relatively high haemorrhage rates, in combination with the low complete recanalization rates, demonstrate the limitations of treatment solely with pharmacological agents. Especially in carotid terminus or basilar artery occlusions with large clot burden, the procedure takes time and may not achieve meaningful recanalization. Platelet-rich clots, old clots and calcified clots or fat emboli cannot be addressed. These factors substantiate the

assumption that faster and more complete reperfusion will lead to better long-term outcomes for acute stroke patients. Endovascular therapy promises higher recanalization rates because of the obvious advantages over IV rtPA, which are site specific, reduction of drug amount and potentially longer treatment windows.

Nevertheless, the potential benefit of endovascular therapy must be weighed against the potential risk of the procedure. Intraarterial (IA) stroke therapy has been associated with a 5–7% risk for clinically significant procedural complications (delzeppo G. J. 1998, Furlan A. 1999) and a 6–15% risk for symptomatic ICH (Smith W. S. 2002). Patients with minor strokes, who are treated with IV rtPA, have an 82% chance of a good outcome and the risk of symptomatic hemorrhage and death is 3% and 1%, respectively (The NINDS Stroke study Investigators, 2005).

Such patients are quite unlikely to benefit from endovascular stroke therapy, as they often show no vessel occlusion on cerebral angiograms (Fisher U. 2005). Most stroke centers offer IA stroke therapy only to patients with major strokes. Different strategies of endovascular therapy are offered to reach reperfusion of occluded vessels in acute stroke. Most techniques focus on removing and dissolving the occlusive thrombus to reestablish antegrade flow, but some use the collateral vessel system of the brain to augment flow retrogradely. The different interventional methods are summarized in Table 4.2.

Table 4.2. Strategies of endovascular stroke therapy

Strategies of endovascular stroke therapy			
Interventional method	Endovascular device used	Reperfusion	
		Ante-grade	Retro-grade
Intraarterial thrombolysis	Microcatheter	X	
Mechanical thrombus disruption	Microguidewire, laser	X	
Percutaneous transluminal angioplasty	Microcatheter with balloon for dilatation	X	
Aspiration of thrombus	Guiding catheter	X	
Endovascular thrombectomy	Clot retriever	X	
Augmented fibrinolysis	Ultrasound	X	
Thrombus entrapment	Stent	X	
Temporary endovascular bypass	Stent		x
Flow augmentation	Balloon catheter		x
Transarterial or transvenous retrograde reperfusion	Balloon catheter		

INTRA-ARTERIAL THROMBOLYSIS

The major theoretic advantage of IA thrombolysis over IV thrombolysis is that microcatheter techniques allow direct access to the occluded intracranial vessel and by penetrating the thrombus with the microcatheter the fibrinolytic agent can be infused into the thrombus. This permits a smaller dose of fibrinolytic agent to reach a higher local concentration than reached by systemic infusion and ideally allows more complete recanalization. Additionally, complications from systemic fibrinolytic effects, including ICH, can theoretically be reduced. Thus, the treatment window for endovascular techniques can be extended beyond the typical IV window by 3–6 h, which is important because of the low number of patients who present during the 3-h time window. The first reports about IA administration of thrombolytic agents, mainly in the posterior circulation, were published in the 1980s. An overall recanalization rate of 60% and a mortality rate of 90% in nonrecanalized patients versus 31% in at least partially recanalized patients were found (Zeumer H. 1983, Hacke W. 1988). Multiple non-randomized case series reported successful use of IA thrombolysis. A review of patients treated with urokinase, showed recanalization rates of >75% when additional endovascular techniques (such as mechanical fragmentation of the thrombus, thromboaspiration, percutaneous transluminal angioplasty, and implantation of stents) were used (Nogueira R. J. 2008). Combined IV and IA thrombolysis Several studies have evaluated the feasibility, safety and efficacy of combined IV rtPA with IA thrombolysis in patients with acute stroke. This approach has the potential of combining the advantages of IV rtPA (fast and easy to use) with the advantages of IA therapy (directed therapy, titrated dosing, mechanical aids to recanalization and higher rates of recanalization), thus improving the speed and frequency of recanalization. In the Emergency Management of Stroke (EMS) Bridging Trial, no difference in the 7–10-day or 3-month outcomes between the IV rtPA or IV placebo followed by immediate intra-arterial therapy with rtPA group were found, but there were more deaths in the IV/ IA group (Lewandowski C. A. 1999). The Interventional Management of Stroke (IMS I) Study investigated a combined IV and IA approach to recanalization in patients with ischemic stroke (IMS study Investigators, 2004). The 90-day mortality rate in IMS I subjects (16%) was numerically lower, but not statistically different from the mortality rate of the placebo (24%) or rtPA treated subjects (21%) in the NINDS-rtPA-Stroke-Trial.

MECHANICAL THROMBECTOMY

When thrombolysis is ineffective or is not considered as a viable option, mechanical devices are used to recanalize the occluded cerebral vessel to restore the flow (IMS 2007). One such device, the Merci Retriever™ (Concentric Medical, Mountain View, CA), consists of a flexible corkscrew-shaped tapered nitinol wire with 5 helical loops that can be threaded in the thrombus, which is then removed by traction (Smith W. S. 2006). The first prospective study that examined a device-based treatment option for ischemic stroke showed a 43% recanalization rate with the device alone and a 64% recanalization rate with additional intraarterial administration of t-PA (Smith W. S. 2008). The Catch System™ (BALT Extrusion, Montmorency, France) is a tiny wire basket that retrieves the thrombus

(Brekenfeld C. 2008). Another useful new technique has been developed in which the clot is simultaneously aspirated from the vessel as it is being extracted. The Phenox Clot Retriever™ (Phenox, Bochum, Germany) consists of a highly flexible core wire compound resembling a pipe cleaner with perpendicularly oriented polyamide microfilaments that create an attenuated palisade. It is deployed distal to the clot and is slowly pulled back under continuous aspiration via the guiding catheter.

The BONnet™ (Phenox, Bochum, Germany) consists of a self-expanding nitinol braiding with polyamide filaments passing through the interior to enlarge the surface area and enable better fixation of the thrombus mass (Liebig T. 2008). The Penumbra System™ (Penumbra, Alameda, CA) has been FDA-approved as a dual approach to clot extraction using aspiration and debulking of the thrombus to reduce or eliminate the clot burden (Natrajan S. K. 2009, PPST 2009). The system includes reperfusion microcatheters that are connected to an aspiration pump through an aspiration tube. A separator is advanced and retracted within the lumen of the reperfusion catheter to debulk the clot, followed by clot retrieval with a ring device that engages the thrombus by capturing it in clasps with a cylinder that is then withdrawn. The recanalization rate is more than 80% and the device has an excellent safety profile (<3% procedural serious adverse events) (Struffert T. 2009). This system has the potential of reopening a vessel without the use of thrombolytics, thus offering dual options for recanalization via a single access platform, and decreases the need to blindly penetrate into the occluded vascular segment because it operates from the proximal end of the clot (Nogura R. G. 2009). Percutaneous transluminal angioplasty (PTA) is particularly useful in cases of atherothrombotic disease, in which the residual stenosis may reduce flow sufficiently to lead to rethrombosis (Abu Chebl A. 2005). PTA can be used alone or with subsequent thrombolytic therapy for distal embolization. The largest study of angioplasty for acute stroke showed that recanalization was achieved in 63.9% of thrombolytic-only treated patients, versus 91.2% in the combined thrombolytic plus PTA treatment group. Because of the risk of vessel rupture and distal embolization, this technique is generally reserved for patients whose flow cannot be restored by more conservative methods (Abu Chebl A. 2005). Interventional treatment of acute ischemic stroke with self-expanding stents for flow restoration has been shown to be an effective method for achieving re-canalization. In a prospective study, Stent-Assisted Recanalization in Acute Ischemic Stroke, 100% successful recanalization was demonstrated in 20 patients, with only 1 symptomatic hemorrhage. The disadvantage of this approach is the implantation of a permanent prosthesis and the need for continuous antiplatelet therapy (Levy E. I. 2009, Jahan R. 2010).

Several techniques are used for mechanical clot disruption. The most common is probing the thrombus with a microguide wire. This technique appears to be useful in facilitating chemical thrombolysis. Alternatively, a snare (e.g., Amplatz goose-neck microsnare, Microvena) can be used for multiple passes through the occlusion to disrupt the thrombus (Berguie M. 2006). A snare can also be used for clot retrieval, mostly in situations in which the clot has a firm consistency or contains solid material. Two devices that use laser technologies have been used to disrupt intracranial clots. The EPAR (Endovaxis, Belmont, CA, USA) is a mechanical clot-fragmentation device based on laser technology. The photonic energy is converted to acoustic energy at the fiberoptic tip through creation of micro cavitation bubbles, causing emulsification of the thrombus which is a mechanical thrombolysis and not a direct laser-induced ablation. In a pilot study with 34 patients, vessel

recanalization occurred in 61.1%. There were ICHs in 5.9% and the overall mortality rate was 38.2% (Berlis A. 2004).

The LaTIS laser device (LaTIS, Minneapolis, MN, USA) uses the slow injection of contrast material as a 'light pipe' to carry the energy from the catheter to the embolus. A safety and feasibility trial was stopped because the device could not be deployed to the level of the occlusion in 2 of the first 5 patients.

INTRA-ARTERIAL (IA) MECHANICAL THERAPIES

The limitations to IA lysis are related to clot characteristics. The response to the pharmacological agent may vary according to the source and type of clot. White platelet rich clots are more resistant to lytics than fresh red blood cell rich clots. De novo cardiac clots and paradoxical venous clots respond better than calcified clots from atherosclerotic plaques. The therapeutic approach concerning these limitations changed dramatically in 2004 with the US Food and Drug Administration approval of the first mechanical clot-retriever (Smith W. S. 2005). The mechanical fragmentation of a clot increases the surface area accessible to fibrinolytic agents thus increases the speed of thrombolysis. The use of chemical thrombolytics is lower, or can be avoided, particularly in patients with contraindications to thrombolytic therapy. The treatment window can be exceeded beyond the limit of 6–8 h. Clot-retrieval devices also may be more efficient in catching mature embolic clots and emboli composed of cholesterol, calcium or other debris from atherosclerotic lesions (Smith W. S., 2005 and 2008, Noguiera R. G. 2009).

SURGICAL INTERVENTIONS

Surgical reconstruction of the cervical carotid artery may be indicated in patients with transient ischemic attack but who are otherwise in good neurologic condition. Another option, microsurgical embolectomy of intracranial vessels, is an invasive procedure involving craniotomy, opening the occluded artery to remove the clot, and reperfusion of the affected area. However, because the procedure is performed through the subarachnoid space via a small craniotomy with a minimal skin incision, it is relatively safe. This can be a last therapeutic option for those patients who are ineligible to receive intravenous or intraarterial thrombolysis, or in the case of failed mechanical thrombectomy. Again, there is a restrictive time window of less than 6 hours from stroke onset for the intervention to be effective. Another surgical procedure utilized in ischemic stroke patients is brain decompression. In the case of major vessel occlusion, large cerebral infarcts is developed, commonly associated with rapidly progressing malignant cerebral edema (Hofmeijer J. 2004). This leads to compromised neuronal metabolism, decreased cerebral perfusion, and impaired oxygenation. In addition, shifting of intracranial contents and transtentorial herniation, is the leading cause of death in these patients (Hacke W. 1996). Because this kind of cerebral edema is refractory, i.e., not amenable to other forms of treatment, brain decompression is considered a therapy of last resort. Decompressive hemicraniectomy with duraplasty is a complex procedure in which a part of the skull vault is removed and the dura is opened to give more room for the

expanding brain. Then, a Silastic sheet, watertight Lyodura, or pericranial grafts are placed over the craniectomy for brain protection. This procedure has been shown to result in increased cerebral perfusion, better survival, improved neurological outcomes, and a reduction in the volume of the infarction (Adams H. P. 2007, Schwab S. 2007). Another, more invasive craniotomy involves resection of infarcted tissue and/or uncal resection. Resection of dead tissue makes room for reperfusion of live tissue in the affected area. Both types of procedures appear to yield satisfactory outcome results.

The disadvantages of the mechanical approaches include the difficulty of navigating devices into the intracranial circulation, excessive trauma to the vasculature (potentially leading to vasospasms, vessel dissection, perforation or rupture) and fragmented thrombus causing distal embolization into previously unaffected territories.

PERCUTANEOUS TRANSLUMINAL ANGIOPLASTY

The feasibility and high efficacy of percutaneous transluminal angioplasty (PTA) in acute stroke has been shown (Ueda T. 1997). Partial or complete recanalization could be achieved in 91% of patients treated with direct PTA versus 64% treated with thrombolytic therapy alone. ICH was seen in 3% versus 19%, and good outcome occurred in 73% versus 50% of the patients, respectively (Nakano S. 2002). 92% recanalization was achieved with the use of balloon angioplasty and adjuvant low-dose eptifibatide and/or thrombolytics with 42% good outcomes (Nogueira R. G. 2008). PTA may also be particularly useful in the cases of atherothrombotic disease, in which the residual stenosis may reduce flow sufficiently to lead to rethrombosis (Quereshi A. I. 2002).

ENDOVASCULAR THROMBOASPIRATION

The use of large 7 to 8-F guide catheter with syringe suction is a simple approach that has been used to remove occlusive thrombus and restore flow in symptomatic acute internal carotid artery occlusion. The guide catheter is slowly withdrawn to facilitate more complete removal of the thrombus (Breckenfield C. 2005). Suction thrombectomy or thromboaspiration through either a microcatheter or a guiding catheter may be an option for a fresh clot. Aspiration devices cause fewer embolic events and vasospasm, but can be difficult to navigate into the intracranial circulation.

The Penumbra System (Penumbra, Alameda, CA, USA) uses a microcatheter and a separator, which is advanced and retracted through the catheter to dislodge the clot and a suction device removes it. This system was initially tested in a pilot trial, where 23 subjects were enrolled. Recanalization before IA lysis was achieved in all treated cases (48%). Good outcome at 30 days was demonstrated in 45% of patients. The mortality rate was 45%. The use of adjunctive IA thrombolytic therapy was associated with a higher incidence of hemorrhage (Bose A. 2008).

The Angiojet (Possis Medical, Minneapolis, MN, USA) is an endovascular rheolytic thrombectomy device that combines local vortex suction, created by high-pressure saline jets, with mechanical disruption. The generated clot fragments are then sucked into the 4- or 5-F

access catheter, which is not flexible enough to allow its navigation into the intracranial arteries. The NeuroJet (Possis Medical) has been designed specifically for intracranial navigation. Unfortunately, vessel dissections and the inability to navigate through the carotid siphon were noted in a pilot study, and the trial was discontinued (Molina C. A. 2005).

ENDOVASCULAR THROMBECTOMY

Endovascular thrombectomy means the extraction of the thrombus with the help of an endovascular tool through a catheter, and should provide rapid recanalization and flow restoration with a reduced risk of distal embolic complications. It is used alone or in conjunction with thrombolytic drugs. The limitations of these devices are intracranial navigation and capture of thrombus within the tortuous cerebral vasculature, and the risk of vessel wall damage or perforation. Some of the devices grasp the thrombus and apply force proximally; others are basket like devices and use a distal approach to the clot.

The Merci Retrieval System (Concentric Medical, Mountain View, CA, USA) is a nitinol wire with an imprinted shape memory effect covered with a platinum coil to improve the visibility under fluoroscopy. It is inserted through a braided microcatheter to a position distal to the thrombus. After release from the microcatheter, it assumes a shape similar to a cork screw and has to be slowly pulled back under continuous aspiration via a balloon guiding catheter. More than 10,000 patients have been treated with this device. Currently the third-generation devices (V series) are used. With a 4.3F distal access catheter (DAC) an additional coaxial support can be added to the system, resulting in improved deliverability with the potential for simultaneous thrombo aspiration or IA thrombolysis. The Merci embolectomy system has been studied in the MERCI and Multi MERCI trials, where a recanalization up to 55% of treatable vessels was reported, and in 68% after adjunctive therapy (IA rtPA, mechanical). Clinically significant procedural complications occurred in 5.5% and ICH in 9.8% of patients (Bose A. 2008, Smith W. S. 2008). As an example how clot-retrievers are supposed to work the Merci retriever is shown in Figure 4.3.

The Phenox Clot Retriever (Phenox, Bochum, Germany) has been CE marked for the treatment of acute stroke in Europe since. It consists of a highly flexible nitinol/platinum-alloy compound core wire surrounded by a palisade of perpendicularly oriented stiff polyamide microfilaments trimmed in a conical shape. It is introduced into the target vessel through a microcatheter deployed distal to the thrombus and slowly pulled back under continuous aspiration via the guiding catheter. The smallest version of the device is used to recanalize distal MCA branches. Recently the second generation of the device (Phenox Clot Retriever CAGE), which incorporates a nitinol cage into the previous design, was constructed for the treatment of thrombi with firmer consistency. Recanalization was achieved in 56.3% of 45 patients with stroke with no device-related morbidity and mortality (Henkes H. 2006). The Neuronet device (Abbot Vascular, Temecula, CA, USA) has also been used successfully to retrieve intracranial clots.

It is a microguidewire-based laser-cut nitinol basket open proximally with the crisscrossing basket portion tapering to a shapable platinum-tipped wire (Mayer T. E. 2002). Very similar is the Catch (Balt Extrusion, Montmorency, France) device, a derivative of a self-expanding braided nitinol stent.

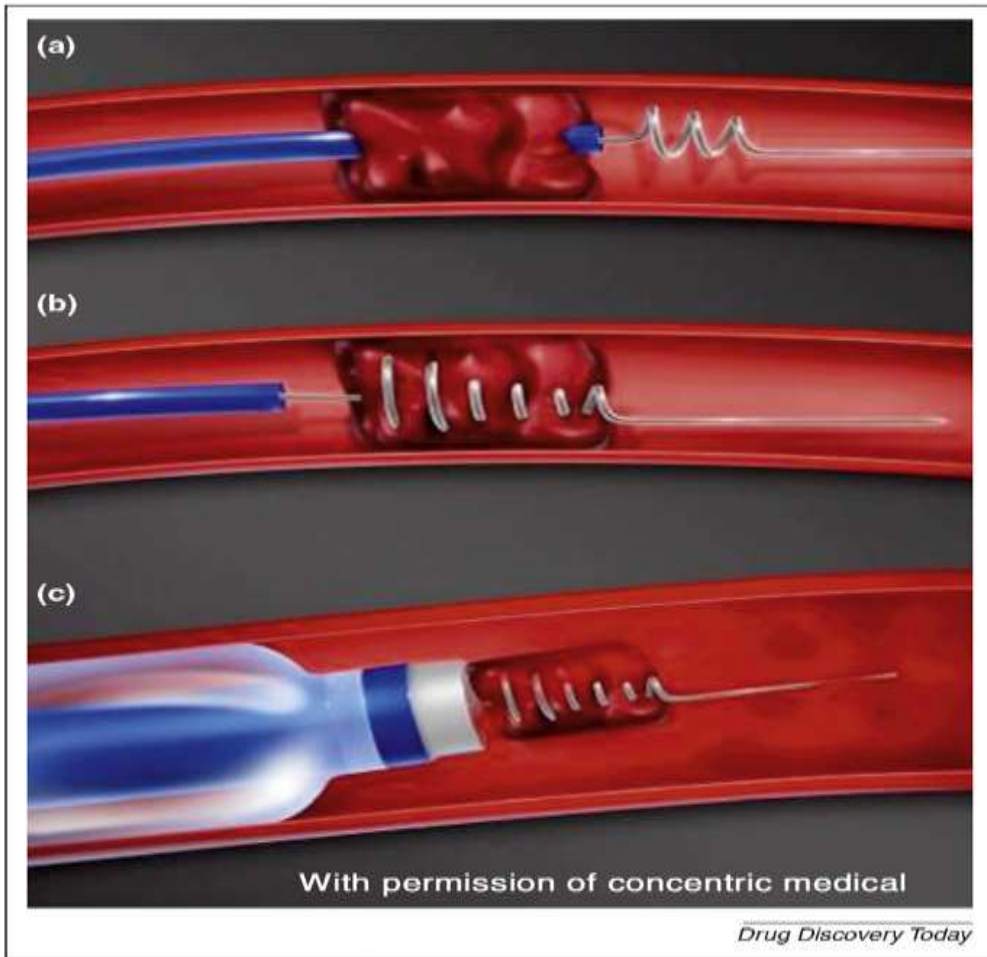


Figure 4.3. *Endovascular Therapy for acute stroke*: The principle of clot retrieving. The Merci System. Endovascular therapy for acute stroke is always performed under general anesthesia. First the MerciBalloon Guide Catheter is placed in the common or internal carotid artery for anterior circulation occlusion. Using standard cerebral catheterization techniques, a microcatheter is guided then through the guide catheter into the occluded vessel and passed beyond the thrombus (a). The Merci Retriever is now advanced through the microcatheter, and several of the helical loops are deployed distal to the thrombus. The Merci Retriever is then retracted to the face of the thrombus, and the proximal loops are deployed within the thrombus (b). The balloon of the Merci Balloon Guide Catheter has to be inflated now to arrest orthograde blood flow during removal of the thrombus. At least the Merci Retriever with the ensnared thrombus and the microcatheter are withdrawn together into the balloon guide catheter lumen. Continuous aspiration is applied to the guide catheter to promote complete evacuation of the thrombus (c). Once a retrieval attempt is completed, the balloon of the balloon guide catheter is deflated to re-establish flow. If the clot could not be removed with the retriever, the procedure can be repeated up to 5 times.

It is a nitinol basket, closed at its distal end and anchored with a pair of nitinol wires for insertion and withdrawal at the proximal end (Chapot R. 2005).

A comparison between the Merci and the Catch devices in an animal model has demonstrated superiority of the Merci retriever, which resulted in higher rates of overall

recanalization (90% versus 70%), higher chances of recanalization at the first attempt, and a lower rate of thrombus fragmentation/distal embolization (Breckenfield C. 2008).

The Alligator Retrieval Device (Chestnut Medical Technologies, Menlo Park, CA, USA) is a retriever with 4 small grasping jaws attached to the tip of a flexible wire. This device has been used to treat 6 patients with intracranial clots (predominantly MCA), resulting in rapid clot removal and clinical improvement in all patients. In limited experience it provided a rapid, safe, and effective means for achieving revascularization (Kerber C. W. 2007). The In-Time Retriever (Boston Scientific, Natick, MA, USA) has 4–6 wire loops and tends to bow when opened but has no specific opening to capture the embolus. This device has been successfully used in case studies (Bergui M. 2006). The performance of 5 different embolectomy systems was recently evaluated using an in vitro pulsatile flow model. The Merci, Catch, and Phenox retrievers were equally able to mobilize and remove most thrombi, whereas the In-Time and Attractor devices achieved only partial thrombus removal at best, and with considerable difficulty during initial thrombus penetration and placement of the device (Liebig T 2008).

AUGMENTED FIBRINOLYSIS

The MicroLysUS infusion catheter (EKOS, Bothell, WA, USA) is a microcatheter with a piezoelectric element at its distal tip, which creates a microenvironment of ultrasonic vibration to facilitate thrombolysis. This is achieved by a combination of a noncavitating sonography, which reversibly separates fibrin strands, and acoustic streaming, which increases fluid permeation, resulting in increased drug-thrombus surface interaction. In a pilot study 14 patients were treated with IA rtPA or reteplase infusion through the EKOS microcatheter and simultaneous sonography transmission for 60 min. Recanalization was achieved in 57% of patients and good clinical outcome in 43%. The mortality rate was 36%. A comparison of angiographic outcomes between subjects treated in the IMS-II trial with the EKOS catheter and IMS-I subjects treated with the standard microcatheter demonstrated recanalization in 73% versus 56% respectively. This device is being investigated further in the IMS-III trial (Nogueira R. G. 2008).

THROMBUS ENTRAPMENT

Stent placement of an acutely occluded intracranial vessel may provide fast recanalization by entrapping the thrombus between the stent and the vessel wall. This may be followed by thrombus dissolution via either endogenous or pharmacologic thrombolysis (Nogueira R. G. 2008). Treatment with balloon-expandable stents showed a recanalization rate of 79% and no symptomatic ICHs (Levi E. I. 2006). Multiple intracranial self-expandable stents are used in acute stroke therapy, although primarily designed for the treatment of wide neck aneurysms: the Neuroform (Boston Scientific), the Enterprise (Cordis, Miami Lakes, FL, USA), the LEO (Balt Extrusion, Montmorency, France) and the Solitaire/Solo stent (ev3 Endovascular, Plymouth, MN, USA). The Wingspan stent (Boston Scientific) is approved for the treatment of intracranial atherosclerotic lesions. Using these stents during stroke

treatment, Gp IIb/IIIa inhibitors are administered intra- or immediately post procedurally to avoid acute in-stent thrombosis. Isolated case reports of successful intracranial recanalization with these stents have been reported (Fitzesimmonis B. F. 2006). The Pharos Vitesse stent (Micrus Endovascular), the only balloon-expandable stent approved for intracranial use, was used successfully in 4 patients with acute strokes (Killer M. et al. unpublished). The prospective 'First Food and Drug Administration-Approved Prospective Trial of Primary Intracranial Stenting for Acute Stroke' trial, called SARIS (Stent-Assisted Recanalization in Acute Ischemic Stroke) suggested primary intracranial stenting for acute stroke may be a valuable addition to the stroke treatment armamentarium (Levi E. I. 2009).

TEMPORARY ENDOVASCULAR BYPASS

The need for an aggressive antithrombotic therapy after stent implantation remains one of the major limitations to its use in acute stroke. However, the advent of closed-cell stents has allowed resheathing and removal of the stent after recanalization. The need for post-treatment dual antiplatelet therapy, which could potentially increase the risk of hemorrhagic conversion of the infarct, is not necessary. Recently the preliminary data about the use of the Solitaire/SOLO device as a temporary endovascular bypass and clot retriever were presented (Liebig T. et al. unpublished). A clinical case in which partial deployment of an Enterprise stent resulted in immediate recanalization of an occluded MCA that was refractory to IV and IA rtPA, IA abciximab, and mechanical manipulation, confirmed this therapeutic concept (Hussain M. S. 2010). The currently newest available device is the TrevoTM System (Concentric Medical, Mountain View, CA, USA), an innovative, easy-to-use thrombus retrieval system. The main component of the Trevo System is a stentrieverTM, a new generation of retrieval devices which is able to restore flow in the occluded brain territory and remove the clot under aspiration with the guiding catheter. The first 24 cases, recently been performed in Europe, showed promising results (Liebig T. et al. unpublished). The alternative reperfusion strategies use the collateral vasculature of the brain as well as retrograde and reversed flow. Retrograde reperfusion and flow reversal are experimental treatment techniques that cause total reversal of the cerebral circulation and perfusion of the venous system with arterial blood into the capillary bed, which is then physiologically proximal to the occluded artery.

FLOW AUGMENTATION

Global reperfusion or flow augmentation tries to increase cerebral blood flow (CBF) to perfuse the brain tissue distal to the occlusive thrombus via leptomeningeal collaterals. This retrograde reperfusion strategy may potentially lead to better recanalization rates when used as an adjunct to antegrade reperfusion treatments. The increase in CBF should result in the better delivery of thrombolytic drugs to the occlusion site, theoretically leading to better recanalization with systemic thrombolysis. Flow augmentation can be achieved mechanically with the NeuroFlo device (CoAxia, Maple Grove, MN, USA). The NeuroFlo device is a dual balloon catheter designed for partial occlusion of the aorta above and below the origin of the

renal arteries. It is intended to increase blood flow to the brain and thus potentially reduce the damage caused by stroke. The recently completed SENTIS Phase III trial (Safety and Efficacy of NeuroFlo Technology in Ischemic Stroke) assesses the safety and efficacy of the NeuroFlo™ catheter for use in patients with ischemic stroke. The results are not yet published. Another ongoing ‘Study to Evaluate the Effects of the Neuroflo Device in People Who Have Had a Stroke’ investigates the feasibility and safety of the NeuroFlo™ in patients with persistent arterial occlusion after attempted thrombectomy with the Merci1 Retriever System. Delayed symptomatic reocclusion after initial endovascular stroke therapy can lead to sudden clinical deterioration and has been linked to poor clinical outcomes (Hussain M. S. 2010). The rate of reocclusion after endovascular treatments is 18% (Janjua N. 2008). This may be underestimated as many patients present with large clinical deficits at time of presentation and explain the low rate of good clinical outcome despite of a high rate of revascularization.

REPERFUSION THERAPIES

The advantages of pharmacological thrombolysis over mechanical means are: The drugs are easier to administer and – ‘Time is Brain’: they can be started faster. Future newer generation lytics and platelet inhibitors may be even faster, effective and specific. The disadvantages are that often no success can be achieved, and although the therapy can be initiated quickly, the effect occurs slowly and the risk of local and systemic hemorrhagic complications is evident. The major disadvantages of an invasive procedure are additional risks and high costs compared with IV rtPA that are weighted against achieving higher reperfusion rates. The current approach to acute endovascular stroke therapy is the use of mechanical clot-retrievers in combination with pharmacological agents. Future trials will address that and explore combined treatment strategies with neuroprotectants, antithrombotics, and supplementary mechanical strategies.

THROMBOLYTIC AGENTS

The critical time period during which this volume of brain tissue is at risk is referred to as the “window of opportunity”. At present, intravenous administration of tissue plasminogen activator (t-PA) within 3 h of symptom onset is the only US FDA-approved treatment available to re-establish cerebral blood flow. Early reperfusion with t-PA increases recovery from stroke symptoms by ~30%, with a low rate of serious complications. This will not only rescue neuronal and glial cells within the penumbra, but also glial cells from the central ischemic core zone, thereby limiting the size of infarcted tissue (Turner R. 2007). However, despite the beneficial outcome of early reperfusion, only 3%–8.5% of eligible stroke patients receive t-PA because of the possibility of intracranial hemorrhage (Bambour K. Z. 2006). The American Heart Association recently suggested that selected patients may benefit from t-PA up to 4.5 h after stroke, but some counter that the risk of hemorrhagic complications beyond 3 h following ischemia is significantly greater due to breakdown of the BBB (Sue E. J. 2008). In addition, the increase in cerebrovascular permeability results in diffusion of t-PA to the

brain parenchyma (Yepes M. 2003), causing neurotoxicity (Gotto H. 2007). To extend treatment availability to patients with stroke and to reduce the potential for intracranial hemorrhage, intraarterial thrombolysis is a viable option for those who present for medical treatment in the 3–6 hour time window (Zaidat O. O. 2002). The strategy behind intraarterial administration is rapid local delivery of a thrombolytic agent through a microcatheter placed near the site of occlusion. This leads to improved recanalization and reduced hemorrhagic complications due to lower doses of thrombolytic agent required to resume blood flow (Rha J. H. 2007). The disadvantages include the relative complexity of the procedure, delays in initiation of treatment, required technical expertise, and the invasiveness of the procedure (Nogueira R. G. 2009). To address the above issues, particularly to compensate for the delay in receiving the treatment, a combination therapy of intravenous and intraarterial thrombolysis has been tested. The initial partial recanalization with intravenous injection of t-PA is followed by complete recanalization via direct intraarterial delivery (Lawendowski C. A. 1997).

PROMISING DIRECTIONS AND POTENTIAL TARGETS IN NEUROPROTECTANT DEVELOPMENT

Despite past shortcomings in neuroprotectant development, multiple pharmacologic agents are currently under investigation in advanced phases of clinical trial development and appear promising based on early phase results. This includes Arundic Acid (ONO-2506), an astrocyte modulator, and minocycline, an inhibitor of microglia.

MODULATING ASTROCYTE ACTIVITY

While neuroprotection research has been largely neurocentric in nature and failed to emphasize the prominent role of glia in both homeostasis and injury response, the suggestion has been made that preservation of neuronal survival and function may not be possible without significant regulation of the microenvironment by astrocytes. Astrocytes, frequently described as “housekeeping cells of the nervous system”, are involved in regulating blood-brain barrier (BBB) integrity, removal of metabolic waste products, release of neural growth factors, and uptake of excess neurotransmitter release during synaptic activity. As such, astrocyte modulation represents a promising strategy for future therapeutic development for ischemic stroke (Takano T. 2009). The effect of ischemia on astrocytes is widespread in that signaling amongst astrocytes, as well as between astrocytes and neurons or microglial cells, is likely altered in addition to the potential for necrotic or apoptotic cellular death. This disrupted signaling likely has significant consequences on the control of basic physiologic functions including cerebral blood flow, glutamate uptake by astrocytes, and the ability of astrocytes to serve as an energy reserve during ischemia (Bambrick L. 2004, Carmignoto G. 2010, Rosi D. J. 2007, Zonta M. 2003). Perhaps the hallmark event involving astrocytes following neural injury, and specifically ischemia, is the development of reactive gliosis and associated morphologic changes and altered gene expression. One of the challenges in modulating astrocyte activity, and associated gliosis, for therapeutic benefit is the emerging

evidence that astrocytes have both protective and detrimental roles, potentially a result of multiple astrocyte subtypes (Zamanian J. L. 2012). Recent work by Zamanian, *et al.* have illustrated this concept in that ischemia appears to promote a protective astrocytic response whereas neuroinflammation induced by lipopolysaccharide (LPS) produces a potentially detrimental phenotype.

Notably, these two different stimuli each induced significantly altered gene expression yet the expression shared between stimuli was less than 50%, furthering the notion of a stimulus-specific astrocyte response or multiple astrocyte subtypes (Zamanian J. L. 2012). The influence of astrocytes on outcome following neurologic injury, and the potential for conflicting roles (beneficial or detrimental), has been seen clearly in a variety of preclinical models including ischemia, experimental autoimmune encephalitis (EAE), and spinal cord injury to name a few. In a preclinical model of ischemia, Li and colleagues demonstrated a protective role of astrocytes by knocking out glial fibrillary acidic protein (GFAP) and vimentin.

Deletion of this combination of intermediate filaments, characteristic of astrocytes, resulted in infarct volumes two to three times larger than in wild-type animals (Li L. 2008). In spinal cord injury, a dual role of astrocyte reactivity has been observed in which initially, astrocytes appear to modulate inflammation and contain inflammatory cells to the damaged region.

On the other hand, astrocytes are the primary component of the glial scar that prevents axonal re-growth in the chronic period post-injury (Okada S. 2006). In a model of demyelinating disease, experimental autoimmune encephalitis (EAE), targeted genetic removal of reactive astrocytes resulted in diminished perivascular scar formation and increased leukocyte infiltration into brain parenchyma. The exacerbated inflammatory response was associated with a detrimental outcome (Voskuhl R. R. 2009).

Despite the expanded understanding of astrocyte roles in homeostasis and disease, clear knowledge gaps remain. This includes more clear elucidation of the timing of beneficial versus detrimental responses to injury, how these responses can be pharmacologically manipulated, and how the astrocyte response is altered in association with disease comorbidities and the aging process. Our laboratory and others have begun to explore these factors with the hope of improving the understanding of neural injury in order to develop future therapeutics (Cotrino M. L. 2002, Dinapoli V. A. 2010). One potentially promising therapeutic that alters astrocyte activation, and synthesis of S-100B, that has advanced to clinical trials is (*R*)-(-)-2-propyloctanoic acid (ONO-2506). S-100B is expressed by reactive astrocytes throughout the penumbral region following ischemia and leads to nitric oxide release from the astrocyte, potentiating neuronal cell death (Teteshi N. 2002). In an early phase clinical trial, ONO-2506, also known as arundic acid, proved safe and produced a trend towards functional improvement based on the National Institutes of Health Stroke Scale (NIHSS) (Pettigrew L. C. 2006).

INHIBITING EFFECTS OF MICROGLIA

Much like astrocytes, microglial modulation may represent a promising therapeutic target for ischemic stroke based on the progression of compounds such as minocycline in clinical trials.

Microglial activation, a hallmark of the inflammatory response within the central nervous system post-stroke, has been described as both beneficial and detrimental in nature (Yenari M. A. 2010). Ablation of Mac-2 positive microglia, a marker of activated microglia, resulted in enhanced infarct volume and altered temporality of pro-inflammatory cytokine release. These changes were associated with a nearly three-fold increase in apoptotic cells and a nearly two-fold decrease in IGF-1, a neurotrophic factor, levels (Lalancetti-Hebert M. 2007). In contrast, numerous other studies have shown an improvement in outcome following ischemia by reducing microglial activation (Hayakawa K. 2008, Sakata H. 2012). This is achieved by administration of minocycline, traditionally used as an antibiotic, which has been associated with diminished Iba-1 expression, an activation marker, by microglia (Hayakawa K. 2008). Importantly, the microglial response post-stroke begins rapidly but takes hours to days to develop fully, potentially resulting in an extended window of therapeutic opportunity (Yenari M. A. 2010). The idea of an extended window, at least in comparison to that used for thrombolytic therapy, is consistent with preclinical findings in which a clinically relevant dosage of minocycline decreased infarct volume at both 4 and 5 h post-stroke while reducing functional deficits with 4 h administration (Xu L. 2004). In addition to the effects on microglia, minocycline has been shown to alter the function of other immune cells previously implicated in ischemic damage such as macrophages (Campbell J. H. 2011). While not previously investigated specifically with regards to ischemia, the effect of minocycline on macrophages may be related to alterations in macrophage recruitment and/or macrophage polarization, a topic that is discussed at greater length in subsequent sections. The effect of minocycline on subsequent inflammatory processes is logical in that microgliosis following injury is associated with not only the acute and rapid activation of microglial cells but also the recruitment of marrow-derived inflammatory cells that migrate to the brain and penetrate the parenchyma (Lalancetti-Hebert, M. 2007). The precise mechanism via which minocycline mediates protection through microglial-mediated effects remains unclear but has been shown to decrease expression of high-mobility group protein B1 (HMGB1) and *in vitro*, reduces oxidative stress via diminished superoxide release (Hayakawa K. 2008, Yenari M. A. 2006). Minocycline may also prove beneficial in other reparative therapies post-stroke, such as stem-cell delivery. Sakata and colleagues demonstrated that minocycline pre-conditioning of neural stem cells prior to transplantation resulted in production of beneficial paracrine factors and diminished graft cell death via upregulation of antioxidant genes associated with Nrf2 (Sakata H. 2012). In early phase clinical trials, minocycline has exhibited similar promise in that it has been proven safe when administered alone or in combination with thrombolytics and also exhibited potential efficacy in these small studies. Namely, NIHSS and modified Rankin Scale (mRS) scores were lower, and Barthel Index (BI) scores higher, in the group given minocycline. This improvement was seen as soon as 7 days post-stroke and persisted at day 30 of follow-up (Lampl Y. 2007). While a large efficacy trial is needed to confirm preliminary findings, minocycline appears promising for the treatment of ischemic stroke (Fagan S. C. 2011). Notably, clinical studies have also shown that minocycline administration results in diminished levels of matrix metalloproteinase-9 (MMP-9) (Switzer J. A. 2011). Preclinical studies have shown a reduction in blood-brain barrier (BBB) permeability following minocycline administration and due to previous associations between BBB permeability and MMP-9 activity, it would appear that these clinical findings indicate a potential effect of minocycline on restoration or maintenance of BBB integrity (Yenari MA 2006, Switzer JA 2011).

NEUROPROTECTIVE THERAPIES

Thrombolysis is likely an effective therapeutic modality because it helps restore blood flow and improve cellular metabolism in ischemic regions not yet irreversibly injured. However, thrombolysis is not targeted at the cellular consequences of ischemia. Therefore, it is essential to add a component that would act as a neuroprotective or prevent the damage caused by reperfusion injury. In fact, the Stroke Progress Review Group, which is charged with assisting the National Institute of Neurological Disorders and Stroke (NINDS) in addressing the Institute's Stroke Research Program, has recommended research on combinations of neuroprotectants or neuroprotectants plus reperfusion therapy. Addition of neuroprotectants would protect neurons, which are susceptible to apoptosis, and could minimize the damage due to reperfusion injury.

MODULATING THE BLOOD-BRAIN BARRIER (BBB)

As focus has gradually shifted from solely the neuron to include glial cells and the associated neurovascular unit, the role of the blood-brain barrier in ischemic injury has received renewed interest. Much of the work involving BBB permeability has focused on potential mechanisms of degradation, such as matrix metalloproteinases, creating a natural target for pharmacologic agents (Lee S. R. 2004a, Lee S. R. 2004b, Lo E. H. 2002, Rossel A. 2008, Zhao B. Q. 2006). In work by Pfefferkorn and colleagues, the authors showed the ability to reduce BBB disruption and improve survival following delayed tPA administration by using BB-94, a matrix metalloproteinase inhibitor (Pfefferkorn, T. 2003). In more recent work using a model of traumatic brain injury, a similar phenomenon was shown using Kollidon VA64, a pharmacological agent described as resealing membranes. Improving membrane integrity resulted in diminished neural injury and improved functional outcome (Mbye L. H. 2012). It is clear that more investigation is required to understand not only the role of BBB permeability in disease but also how to target BBB permeability therapeutically, but studies such as those described herein provide a potential proof-of-concept and perhaps most importantly, further indicate the need for a comprehensive approach to ischemic stroke therapeutic development.

TARGETING INFLAMMATION

As demonstrated by the early phase success of agents currently under investigation, such as minocycline and arundic acid, targeting inflammation may offer promise in the quest for identification of neuroprotective compounds. Inflammation offers many opportunities and targets for modulation, many of which persist for hours to days, sometimes even longer, following injury. Consequently, these targeted therapeutics are more likely to be administered within the window of opportunity for a given pathologic process compared to some of the previously tested agents that target events occurring almost immediately post-stroke. Inflammation, while generally thought of in a negative connotation, is an essential component of recovery and repair-associated processes as well, further complicating experimental

investigations. In this work, we focus on the dual responses associated with inflammation, particularly with regards to macrophage polarization, an emerging topic in both the inflammation and neuroscience literature.

INFLAMMATION: A DELETERIOUS EVENT OR A BENEFICIAL RESPONSE?

The inflammatory process is further complicated due to the fact that various elements can serve a deleterious or a beneficial role in the injury and recovery process, as highlighted earlier in this work with discussion of astrocytes and microglia. Another prime example of this potentially dual response to neural injury is that of macrophages. Macrophages and microglia may contribute to secondary injury processes via production of inflammatory cytokines and reactive oxygen species while also potentially reducing injury through maintenance of the cellular microenvironment by phagocytic clearance of cellular debris and apoptotic cells (David S. 2011, Popovich P. G. 2008). Emerging evidence indicates that many of these inflammatory cell types may never truly exist in a resting state and rather may almost constantly be undergoing changes in state through intermediary forms, evident by both morphologic and functional changes, in response to the cellular environment and signals from the surrounding regions (Pollazi E. 2010). This is perhaps most adequately described, and potentially targeted therapeutically, by macrophages. In the following section, we attempt to highlight some of the recent developments in the macrophage literature, particularly those related to various macrophage subsets and neural injury.

INFLAMMATION

Several inflammatory mediators such as leukocytes, adhesion molecules, acute phase reactants, cytokines and proteases increase in the plasma of patients with cerebral ischemia. The early central inflammatory events include the production of reactive oxygen species (nitric oxide, superoxide) expression of proteolytic enzymes (MMP-9, MMP-2), vasoactive substances (prostaglandins and cyclooxygenases) and vascular adhesion molecules (ICAM-1, P-selectin, L-selectin). Neutrophils and macrophages are considered early contributors to the production of ROS and cytokines, while activation of microglia and proliferation of astrocytes in the ischemic area further promotes the inflammatory response in later stages (Dennis A. 2009). Inflammatory mediators, [such as IL-1 β , IL-6, TNF α , IL-10, transforming growth factor beta (TGF β) and chemokines including MCP-1, macrophage inflammatory protein-1 (MIP-1), keratinocyte-derived chemokine/chemokine (CXC motif) ligand 1/(KC/CXCL1) and fractalkine (CX3CL1)], are also found in increased levels in brain tissue in animal models of stroke.

The expression of IL-1 β mRNA is increased in the ischemic rat brain within hours after the induction of stroke (Liu T. 1993), elevated levels of IL-6 are seen in the plasma and cerebrospinal fluid (CSF) 3–6 h after experimental stroke in mice (Clark W. M. 1999), and levels of pro-inflammatory cytokines such as IL-6, IFN γ , MCP-1 become elevated in the plasma within 6 hours in rodents (Offner H. 2006).

The intercellular adhesion molecule-1 (ICAM-1) antibody, Enlimomab, can reduce the size of stroke when given within 1 hr reperfusion after transient, but not permanent ischemia, in a rat stroke model.

However, its use was associated with significant worsening in the modified Rankin scale and higher mortality at 90 days compared to placebo in a large clinical trial (Enlimomab Group 2001). Blocking neutrophil activation by a recombinant protein inhibitor of the CD11b/CD18 receptor, UK 279,276 (Rovelizumab), within 6 hrs of stroke also failed to show any benefits in one clinical trial which was stopped prematurely. Despite promising results for interleukin (IL)-1 receptor antagonist (RA) in several animal studies, this drug has not been investigated in humans (Banwell V. 2009). FK506 also demonstrates benefits, particularly after transient ischemia, in animal studies and remains a potential target for studies in humans (Mecleod M. R. 2005).

Systemic administration of minocycline is protective in experimental models of focal (Yrjanheikki J. 1998) or global (Yrjanheikki J. 1999) cerebral ischemia. Inhibition of IL-1 β converting enzyme (Harra H. 2007) or deletion of IL-1 β and IL-1 α results in markedly reduced ischemic damage and neuronal death in animal studies (Boutin H. 2001). Deficiency of inflammatory chemokines such as MCP-1 (Huges P. M. 2002) or fractalkine (Soriano S. G. 2002) is associated with less vulnerability to ischemic injury.

Cerebral ischemia activates an inflammatory reaction within hours of the injury. The suppression of inflammation using a variety of drugs has been shown to reduce infarct area in animal studies. Commonly used anti-inflammatory agents are aspirin and the lipid lowering statins.

A combination of aspirin and extended release dipyridamole was found to be more efficacious than aspirin alone for nonfatal stroke by the second European Stroke Prevention Study (ESPS-2) and the European/Australian Stroke Prevention in Reversible Ischemia Trial (ESPRIT) (Deb P. 2007).

In an investigation of the anti-inflammatory and neuroprotective properties of statins, the Stroke Prevention by Aggressive Reduction in Cholesterol Level study showed that treatment with high-dose atorvastatin reduces the risk of stroke in patients with the first attack of stroke and no known coronary artery disease (Gedikli O. 2008). In addition to aspirin and statins, two leukocyte adhesion inhibitors, Enlimomab and LeukArrest, were studied in patients with ischemic stroke. Although Enlimomab was shown to reduce the damage in stroke models, patients who received it experienced significantly more negative effects relative to placebo controls (Cheng Y. D. 2004).

Another neuroprotective approach was explored in the “Intravenous Thrombolysis Plus Hypothermia for Acute Treatment of Ischemic Stroke (ICTuS-L)” study of the effects of induced hypothermia and thrombolysis after acute ischemic stroke. Hypothermia is thought to serve a neuroprotective role in stroke via inhibition of the MIP-3 α -CCR6 inflammatory pathway (Shintani Y. 2011).

Although hypothermia has been shown to confer some protection against damage in cardiac arrest and neonatal hypoxic-ischemic encephalopathy patients (Lundby J. B. 2011, Jacobs S. E. 2011), its effectiveness in acute ischemic stroke has not been definitively demonstrated. Negative consequences, including pneumonia, were seen more frequently in patients who had undergone hypothermia treatment, compared to controls in this study. Disappointingly, there was no significant benefit in mRS between the two trial groups (Hemmen T. M. 2010).

EXCITOTOXICITY AND NMDA GLUTAMATE RECEPTORS

Excessive NMDA (N-methyl D-aspartate) receptor activation has been implicated in the pathophysiology of several neurodegenerative diseases and in stroke. Excitotoxicity has been a widely investigated area in stroke. Ischemic neuronal injury in vitro is dependent on synaptic release of excitatory amino acids and resultant elevation of intracellular free calcium. Even transient exposure to excess excitatory amino acids (EAAs) is toxic to cultured neurons, and alterations in neuronal energy balance increases the vulnerability of neurons to excitotoxic damage even in the presence of physiological concentrations of EAAs (Muir K. W. 1995). Evidence that this process progresses over several hours after the ischemic insult highlights a potential role for neuroprotective strategies administered during the critical window prior to irreversible loss, although the exact duration of this window in humans remains unknown. The action of glutamate on NMDA receptors seems to play an important role in glutamate-mediated toxicity. Compounds that decrease glutamate levels or interfere with its binding to this receptor have been the focus of many studies in this area (Nagai Y. 2008). NMDA antagonists, which have been investigated in stroke include Aptiganel hydrochloride (CNS 1102, Cerestat), dextrorphan and dextromethorphan. Their use was associated with side effects and no clear clinical benefit in clinical trials (Muir K. W. 1995). Phase 2 trials of both oral and IV forms of remacemide hydrochloride in stroke are currently underway. Magnesium ion, which electrophysiologically behaves as a noncompetitive NMDA antagonist, has demonstrated efficacy as a neuroprotective agent in focal and global models of cerebral ischemia. Two preliminary trials of IV magnesium show no evidence of adverse effects (Muir K. W. 1994, Wester P. 1985), and phase 3 trials are currently underway. A phase 2 trial of Selfotel (CGS 19755) in acute stroke patients, reported evidence of a dose-dependent toxicity (Clark W. 1994). Selective NMDA antagonists such as ifenprodil and eliprodil (SL82.0715) have demonstrated preclinical neuroprotective efficacy, and eliprodil is currently being investigated in early phase 3 trials in stroke patients. Other compounds under investigation are 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP), its derivative d-CPPene, and CGS 19755 (Taylor C. P. 1994). Neuroprotection is evident for the presynaptic glutamate release inhibitors 619C89 and ubeluzole when administered within 6 hours of induced ischemia. Both drugs are currently in phase 2 trials in stroke. Partial glycine agonists, such as HA 966, L687414, and 1-aminocyclopropanecarboxylic acid (ACPC), or full antagonists, such as 7-chlorokynurenic acid and its derivatives or ACEA 1021, are effective in stroke models. Glutamate antagonists at other receptor subtypes, such as the AMPA receptors, are under evaluation (Muir K. W. 1995).

MITOCHONDRIAL DYSFUNCTION

The release of multiple apoptogenic proteins from mitochondria has been identified in ischemic and post-ischemic neurons. Results from animal models strongly implicate caspase-dependent and caspase-independent apoptosis and the mitochondrial permeability transition as important contributors to tissue damage, particularly when induced by short periods of temporary focal ischemia (Sims N. R. 2009). Prophylactic administration of oral creatine reduces the size of ischemic brain infarctions in mice. The anti-oxidant Szeto-Schiller peptide

(SS-31) plays an important role in modulating ROS-induced mitochondrial permeability transition and cell death, and has been found to be protective in several *in vitro* and *in vivo* models of ischemia and reperfusion injury. Synthetic triterpenoids (TP) are analogues of oleanolic acid, which exert anti-oxidative effects through stimulation of the antioxidant response element (ARE)–Nrf2–Keap1 signaling pathway, and have been shown to be protective in a rat model of cerebral ischemia (Chatturvedi R. K. 2008).

ANTI-APOPTOTIC AGENTS

Caspase is a group of cysteine proteases that induce apoptotic cascade when activated. Inhibition of caspase activity has been shown to reduce the size of infarction in rodent stroke models (Cheng Y. 1998, Endres M. 1998). However, none of the caspase inhibitors have yet been tested in clinical trials. A 2009 study on the efficacy of erythropoietin as a neuroprotectant yielded negative effects and safety concerns after it was shown that not only did erythropoietin not confer a neuroprotective benefit, but erythropoietin in combination with the current standard of care, rt-PA, increased the risk of death, intracerebral hemorrhage, brain edema, and thromboembolic events (Ehrenreich H. 2009). Thus, future studies should evaluate the effect of putative therapeutic agents in conjunction with the use of rt-PA where appropriate. These studies demonstrate some of the challenges faced in seeking neuroprotective agents to reduce the initial damage wrought by ischemic stroke.

In general, anti-apoptotic mechanisms being evaluated in neuroprotection include strategies to prevent caspase-dependent apoptosis (e.g., caspase inhibitors) or strategies to prevent caspase-independent apoptosis (e.g., PARP inhibitors). Both caspase-dependent and caspase-independent mechanisms of cell death are implicated in focal cerebral ischemia. Increased expression of Fas and of mediators of the extrinsic caspase-dependent pathway have been shown following focal ischemia. Increased expression of caspase-1, -3, -8, and -9, and of cleaved caspase-8, has been observed in the penumbra. The role for apoptosis in ischemia is further supported by reports that the inhibition of caspase-3 reduces infarct size after transient focal ischemia (Ferrer I. 2003). Intranuclear MMP activity facilitates oxidative injury in neurons during early ischemia through the cleavage of PARP-1 and XRCC1, and resultant disturbance of DNA repair mechanisms. Inhibition of MMP with the broad-spectrum inhibitor, BB1101, significantly attenuated ischemia-induced PARP-1 cleavage, and resultant cell death in rat model of focal cerebral ischemia (Yang Y. 2009). The p53-dependent receptor pathway also seems to be involved in stroke induced apoptosis. Injection of netrin-1, a ligand of the receptor uncoordinated gene 5H2 (UNC5H2), was associated with significantly reduced infarct volume at 3 days after focal ischemia in an animal model of stroke, through its inhibition of p53-mediated apoptosis (Wu T. W. 2008). Estrogen has also been shown to prevent Fas-mediated apoptosis in experimental models of stroke (Jia J. 2009).

ANTIOXIDANT THERAPY

Excessive production of reactive oxygen species (ROS) after cerebral ischemia and reperfusion is implicated in brain damage through different cellular and molecular

mechanisms, and it is further aggravated by impaired cellular antioxidant defense systems under ischemic conditions. Upon reperfusion, cells are often in a state of oxidative stress, which results in further tissue injury, known as reperfusion injury (Adibhatla 2008a and 2008b). ROS are usually scavenged by antioxidant enzymes, primarily superoxide dismutase (SOD), by catalyzing the dismutation reaction of the superoxide anion to hydrogen peroxide (H_2O_2).

Catalase and glutathione peroxidase, on the other hand, protect cells from the toxic effects of H_2O_2 by catalyzing its decomposition into water (Margail I. 2005). However, two pharmacodynamic factors impede the straightforward use of native forms of antioxidant enzymes in cerebral ischemia. First, their molecular weight is well below the renal glomerular filtration cutoff, resulting in their rapid clearance from systemic circulation (e.g., $t_{1/2}$ of SOD in rats = 4–8 min, catalase = 8–10 min). Second, they are negatively charged at physiologic pH and therefore do not readily cross cell membranes (Francis J. W. 1997). Transient disruption of BBB permeability might permit some access of exogenous enzymes to ischemic neuronal tissue, but they do not permeate cells; neurons and astrocytes do not appear to take up the native enzyme under normal conditions, and hence cannot neutralize ROS formed intracellularly (Schaller B. 2004). Because of these challenges, animal studies have shown no improvement in cerebral blood flow or neurological recovery with SOD or SOD with catalase (Forsmann M. 1998, Schurer L. 1990).

Different alternatives have been investigated to address these issues: for instance, PEGylation and lecithinization to improve the circulation half-life (Tsubokawa T. 2007) and fusion of enzymes, e.g., SOD, with cell membrane-penetrating peptides like transactivator of transcription (TAT) of human immunodeficiency virus or tetanus toxin fragment to increase its ability to cross the BBB (Kim D. W. 2005). However, there are limitations to these modifications. For example, PEGylated SOD (PEG-SOD) increases the enzyme's stability in circulation from 6 min to 36 h, but it limits the permeability of SOD across cerebral cell membranes and its ability to be taken up by neuronal cells (Veronesse F. M. 2002). Similarly, fusion of different cell-penetrating or cell-specific peptides to target proteins requires a crosslinker via a chemical process that could cause denaturation and loss of activity of the target protein (Morris M. C. 2001).

There is also a concern regarding the possible immune-mediated anaphylactic responses to hybrid proteins given to patients (Francis J. W. 1997). Intravenous delivery of SOD loaded into liposomes has shown partial inhibition of infarct volume, but the instability of liposomes *in vivo* (half-life ~4.2 h) limits the duration of SOD activity and efficacy (Imazumi S. 1990, Sinha J. 2008). Our approach uses biocompatible, biodegradable nanoparticles (NPs) loaded with superoxide dismutase (SOD-NPs) with the aim to reduce the damaging effect of ROS. These NPs are formulated using poly dl-lactide *co*-glycolide (PLGA) with antioxidant enzymes encapsulated. The enzyme encapsulated in NPs is released in active form. Our initial studies demonstrated the neuroprotective effect of SOD-NPs in oxidative stress-challenged human neural cells, whereas SOD in solution and PEG-SOD were ineffective. The mechanism of protective efficacy of SOD- NPs appears to be due to sustained bioavailability of antioxidants and intracellular delivery of antioxidant enzymes (Reddy M. K. 2008). In our first *in vivo* ischemic middle cerebral arterial occlusion rat stroke model (Reddy M. K. 2009), we determined that the most efficient method of delivery of NPs is via intracarotid artery injection. The study with dye-loaded NPs demonstrated NP localization primarily to the

cortex and striatal regions of the ischemic hemisphere, but not to the nonischemic hemisphere.

Our results demonstrated that over 75% of SOD-NP-treated animals showed increased survival and neurological recovery over a time period of 28 days. In addition, a reduction in breakdown of the blood-brain barrier in treatment animals was evidenced by significantly decreased Evans blue leakage in the brain. These results have driven further research into the neuroprotective effects of SOD-NPs, as well as investigation into how we can further abrogate the negative effects of ROS production in ischemic stroke.

The protective effect of SOD-NPs could be due to the direct antioxidant effect of the active enzyme released from the NPs localized in the brain. It is possible that SOD-NPs prevented the cascade of events or acted on several stages in the pathophysiology of reperfusion injury. Breakdown of the BBB is an early event arising from free-radical damage, especially from superoxide radicals generated in endothelial cells during ischemia and reperfusion (Rosenberg G. A. 2007). SOD-NPs localized in the lining of the cerebral vasculature protected the BBB from free-radical injury, which then could have prevented the leukocyte adhesion that otherwise is a common event following reperfusion as well as prevented edema formation (Kuroda S. 1997).

We are investigating further to understand the mechanism of recovery of animals with time. It is quite possible that the conducive conditions created in the brain as a result of the sustained free-radical scavenging effect of SOD released from NPs localized in the brain (Yasui K. 2006) could have played a role in facilitating the process of angiogenesis and neurogenesis in the infarcted brain. Recently, we began working on developing a thromboembolic rat stroke model to mimic the clinical scenario (Zhang Z. 1997). We are now testing how SOD-NPs and catalase-loaded NPs, or combination of these formulations, would perform in the presence of t-PA and whether we can extend the window of t-PA treatment with effective antioxidant delivery.

Therapeutic strategies based on exogenous delivery of the native form of superoxide dismutase (SOD), a free radical scavenger, are limited because of its short half-life (~6 min) *in vivo* and poor permeability across the blood-brain-barrier (BBB). We encapsulated SOD in biodegradable poly(D,L-lactide *co*-glycolide) nanoparticles (SOD-NPs) and tested their efficacy in a rat focal cerebral ischemia-reperfusion injury model. We hypothesized that localized brain delivery of SOD-NPs would sustain the protective effect of SOD by neutralizing the deleterious effects of ROS formed following ischemia-reperfusion. SOD-NPs were administered at the time of reperfusion *via* the intracarotid route to maximize their localization in the brain. Animals receiving SOD-NPs (10,000 U of SOD/kg) demonstrated a 65% reduction in infarct volume, whereas an equivalent dose of SOD in solution (SOD-Sol) increased it by 25% over saline control ($P < 0.001$; data at 6 h following reperfusion). Control NPs alone or mixed with SOD-Sol were ineffective in reducing infarct volume, with results similar to saline control, indicating the protective effect of the encapsulated enzyme. SOD-NPs maintained BBB integrity, thereby preventing edema, reduced the level of ROS formed following reperfusion, and protected neurons from undergoing apoptosis. Animals treated with SOD-NPs demonstrated greater survival than those with saline control (75% vs. 0% at 28 days) and later regained most vital neurological functions. SOD-NPs may be an effective treatment option in conjunction with a thrombolytic agent for stroke patients.—Reddy, M. K., Labhasetwar, V. Nanoparticle-mediated delivery of superoxide dismutase to the brain: an effective strategy to reduce ischemia-reperfusion injury.

GLUTAMATE ANTAGONISTS

Radical scavengers (such as vitamin E, sulfhydryl compounds and nitron spin traps), agents that promote detoxification of ROS (superoxide dismutase, catalase or peroxidase conjugates), and agents that prevent radical generation (e.g., the xanthine oxidase inhibitor allopurinol, nitric oxide synthase inhibitors, or nonsteroidal anti-inflammatory agents and cyclooxygenase-inhibitors) have been studied in stroke. The use of NXY-059 has been associated with clinical benefits in animal models of stroke, and was associated with a significant improvement in the modified Rankin functional scale in the Stroke-Acute Ischemic NXY Treatment-I (SAINT-I) (Lees K. R. 2006). However, these results were not reproduced in the expanded SAINT-II trial (Feuerstein G. Z. 2008, Shuaib A. 2007). Ebselen and the radical scavenger edaravone (MCI- 186, Radicut) were associated with clinical improvement in a small trials (Green A. R. 2005).

Glutamate antagonists are the most studied neuroprotective agents. As mentioned earlier, glutamate is one of the excitatory CNS neurotransmitters and is released excessively during ischemia (Guyot L. L. 2001). Several receptors relevant to neuroprotection, such as NMDA, AMPA, KA, and metabotropic receptors, are activated by glutamate. Since the activation of most of these receptors is associated with calcium influx leading to cell damage, both glutamate receptor antagonism and calcium channel blockers might serve a neuroprotective role. Although glutamate receptor antagonists have shown excellent neuroprotective effects in animal studies, these effects have not been validated in clinical studies. As a result, studies of several NMDA antagonists- Selfotel (CGS19755) (Boast C. A. 1988), Eliprodil (Gotti B. 1988) and Aptiganel (Cerestat, CNS 1102) (Aranowski J. 1994)- have been halted. Calcium channel blockers such as Nimodipine (Horn J. 2000) and Flunarizine also showed no significant benefit versus placebo in clinical trials. Despite these failures and various side effects at both the psychiatric and cardiovascular levels, the possibility of a role for glutamate receptor antagonists in neuroprotection still exists. A phase III trial with YM872, an AMPA receptor antagonist, is ongoing, and seeks to determine its potential efficacy in combination with *t*-PA thrombolysis. No clinical trials with metabotropic glutamate receptor antagonists have been conducted. It was recently reported that SP-8203, an NMDA antagonist, has dual effects that protect cells against excitotoxicity and improve memory in brain ischemic injuries (Noh S. J. 2011), suggesting a potential therapeutic role of SP-8203 in cerebral ischemic injuries. The main cause of failure of many approaches in clinical studies is inadequate dosing and timing of therapeutics, particularly an unrealistic time window derived from animal models that cannot be duplicated in humans (Leker R. R. 2002). The reasons for the failure of the most promising drug in a clinical study (NXY-059 in the SAINT II trial) are that the drug was not given to most patients within a 4- to 6-h time window as was used in animal studies and its inadequate drug levels in the brain (Feur Stein G. Z. 2008). One phase III trial, the FAST-MAG study, which aims to determine the safety of magnesium sulfate when administered acutely in the first minutes after stroke onset, addresses these issues by arming paramedics with tools to obtain consent and administer the experimental therapy within a hyperacute therapeutic window (Saver J. L. 2004). A pilot study for this trial found significantly improved modified Rankin scores (mRS) at day 90 for trial patients, and the study design has contributed a novel experimental strategy that could benefit other researchers in translating pre-clinical study results to field applications. Recognizing the

benefits of pre-hospital neuroprotective agent administration, the Stroke Treatment Academic Industry Roundtable (STAIR) meeting sets recommendations for priorities in stroke research, noting that the hyperacute treatment window could potentiate other interventions and increase the time window for other time-sensitive interventions (Albers G. W. 2011).

ION CHANNEL MODULATORS

As previously discussed, the influx of calcium, sodium and of potassium during the ischemic cascade leads to brain tissue damage (Ohta K. 2001). Therefore, ion channel modulators might have a neuroprotective role. Nimodipine, a calcium channel blocker that dilates intracranial blood vessels and improves the regional blood flow in the margins of the brain infarct, did not show any benefit in clinical trials. Similarly, Fosphenytoin, the sodium channel blocker, was shown to decrease the amplitude of sodium-dependent action potentials and to block voltage- dependent calcium entry into synaptosomes. Again, phase III clinical trials were terminated due to the lack of demonstrated benefit. The potassium channel activator Maxipost also failed at phase III clinical trials, even though it was expected to protect neurons by inducing neuronal hyperpolarization and decreasing the release of excitatory amino acids (Gribkoff V. K. 2001).

REPAIR AND RECOVERY STRATEGIES OF STROKE: TROPHIC FACTORS AND CELL-BASED THERAPIES

No matter how successful acute neuroprotective strategies become, many stroke patients may still fall outside of clinical time windows for effective treatment. Hence, approaches that promote repair and recovery will be essential for an integrated stroke armamentarium. Logically, these approaches can be divided into two categories—pharmacologic or trophic factor treatments that seek to amplify and augment endogenous processes of neurovascular plasticity and recovery and cell based therapies that seek to replace damaged or lost brain cells.

Many approaches that augment endogenous recovery have been explored. Growth factors such as NGF, FGF, GDNF, and BDNF have all met with variable degrees of success in animal models (Semkova and Kriegstein, 1999). But a limiting factor in many of these studies is their relatively large molecular weight, making transport into brain difficult. Some alternatives may yet exist, such as smaller peptides, transnasal delivery of growth factors to bypass the blood-brain barrier (Fletcher et al., 2009), peptidomimetics and compounds that can upregulate endogenous trophic factor production but do not require direct infusion into brain (Zhang and Chopp, 2009).

Additionally, one may also target inhibitory molecules that accumulate in the area surrounding the injury and block neuronal repair, such as chondroitin sulfate proteoglycans, myelin associated proteins, Nogo, and semaphorins (Silver and Miller, 2004). Although stroke-damaged brain usually exhibits varying degrees of spontaneous recovery, there will always be significant areas where endogenous repair is insufficient. In this regard, cell-based therapies may represent a new frontier. To date, a variety of cell sources have been tested,

including neural stem cells, bone-marrow-derived mesenchymal cells, as well endothelial progenitor cells (Koch et al., 2009). Treatments have typically involved direct transplantations into brain parenchyma, catheter infusions into cerebral ventricles, as well as systemic injections into circulating blood. The latter approach is especially intriguing, as complex mechanisms of cell targeting seem to be operational in brain. Infarcted brain tissue upregulates and releases various chemokines, such as SDF-1, to establish gradients that broadly serve to attract stem and precursor cell populations (Madri, 2009). Induced pluripotent stem cells may also provide an alternative cell source to embryonic stem cells (Takahashi and Yamanaka, 2006). One advantage is the theoretical possibility that cell repair can be custom designed to suit specific diseases or individual needs, as described in a flurry of recent studies showing that neurons can be successfully derived from pluripotent cells taken from patients with Huntington's disease, ALS, and spinal muscular atrophy (Dimos et al., 2008; Ebert et al., 2009; Park et al., 2008). Whether similar methods can be applied to stroke victims remains to be tested.

Overall, cell-based therapies in models of experimental stroke suggest that delayed repair is a viable treatment approach. But more work is needed to translate these promising results into effective stroke therapies. Many questions remain, and the precise mechanisms of cell-induced repair are still unclear. For example, do the reported benefits occur because cells are being explicitly replaced or because transplanted cells serve as a source of trophic factor production? How well do transplanted cells survive? Will adjunctive therapies to decrease apoptotic dropout be necessary? If cells survive, how well do they integrate into existing neural or vascular networks? What is the optimum timing for such therapy? What types of cells should be used?

And finally, what subtypes of stroke patients should be selected for robust clinical trial results? As the basic mechanisms of trophic and cell-based repair continue to be defined, an interesting underlying theme has emerged that links the survival of newborn or transplanted cells to the host environment. As discussed in the previous sections, activated vascular cells and microglia may be detrimental to cell survival in the early stages of cerebral ischemia, due to production of cytokines, ROS, and toxic amount of NO. In the recovery phase, however, chronically activated microglia may now secrete beneficial factors such as BDNF and GDNF. Hence, prolonged inflammation can serve biphasic roles—dampening cellular recovery acutely but then enhancing tissue repair later on.

In fact, this principle of biphasic mediators may be broadly applicable in stroke pathophysiology. There are many examples. Overactivation of NMDA receptors induces acute excitotoxicity (Besancon et al., 2008), but without NMDA signaling, chronic neuronal remodeling cannot take place (Young et al., 1999). Matrix metalloproteinases degrade and damage neurovascular substrates in the acute phase (Rosenberg, 2009), but the same proteases are critically important for neurovascular remodeling during the recovery phase (Zhao et al., 2006; Lee et al., 2006).

The intracellular mediator HMGB1 promotes necrosis and expands the core infarct during acute cerebral ischemia (Qiu et al., 2008), but during the repair phase, HMGB1 release from reactive astrocytes promotes angiogenesis and synaptic plasticity (Hayakawa et al., 2010a, 2010b). Altogether, the boundaries between cell death and cell repair may be blurred (Lo, 2008b). Future studies that dissect these biphasic mechanisms should prove fruitful if we are to develop clinically meaningful strategies for both neurovascular protection as well as repair.

MACROPHAGE POLARIZATION AS THERAPEUTIC TOOL

Macrophages have long been recognized as a key element in the inflammatory response, particularly in reference to the invasion of the body by foreign material such as bacteria. Besides their role as immune effector cells, macrophages are perhaps most important for normal homeostatic processes such as cellular turnover and clearance of cellular debris, hence the description of macrophages as the “janitorial” cell of the body. For example, macrophages are intimately involved in the clearance of over 200 billion erythrocytes each day as well as clearance of apoptotic cells. Consequently, macrophages respond to both endogenous stimuli produced through injury as well as to foreign cell types (Mosser D. M. 2008). As such, work in both the nervous system as well as other body systems indicates that macrophages can be shifted to either a pro-inflammatory or anti-inflammatory state depending on disease states and environmental cues. Gaining a better understanding of the processes mediating this phenotypic shift may lead to therapeutic advances (David S. 2011). Perhaps the greatest challenge in this process is the complexity of elucidating these mechanisms *in vivo*. *In vitro* studies allow for rapid stimulation of inflammatory cells with antigens or cytokines but this response often does not translate to *in vivo* work, or is at the very least, often modified in a significant way (Murray P. J. 2011).

M1 VERSUS M2: CLASSICAL VERSUS ALTERNATIVE ACTIVATION

Literature focusing on macrophage polarization and subtypes has followed a similar path to that of T-cell literature and, consequently, macrophages have largely been classified as M1 or M2, a description that persists despite evidence showing greater complexity, particularly amongst the M2 population (Figure 1) (Mosser D. M. 2011). In spite of the potential for additional subtypes, we use the conventional M1 and M2 descriptions for organizational purpose but do explore the potential for various M2 subtypes within this work.

CLASSICALLY ACTIVATED MACROPHAGES (M1)

Classical macrophage activation refers to those designated as effector cells as part of cell-mediated immune responses. In the past, classical activation occurred in response to two primary signals—interferon gamma (IFN γ) and tumor necrosis factor (TNF) (Mosser D. M. 2008). This results in increased secretion of pro-inflammatory cytokines and mediators such as IL-12, IL-23, IL-1 β , TNF- α , reactive oxygen species (ROS), and nitrosylated species (NS), ultimately producing increased microbicidal or tumoricidal capability (David S. 2011). While emerging evidence indicates that other signals may also induce an M1 response, such as certain toll-like receptor (TLR) agonists, the idea remains the same in that M1 macrophages, while vital for host defense, must be carefully regulated due to the possibility of host-tissue damage with unchecked activation. Persistent inflammation and the associated host-tissue damage is a hallmark of numerous autoimmune diseases such as rheumatoid arthritis and inflammatory bowel disease, demonstrating the need for regulation and strict control of activation processes (Mosser D. M. 2007).

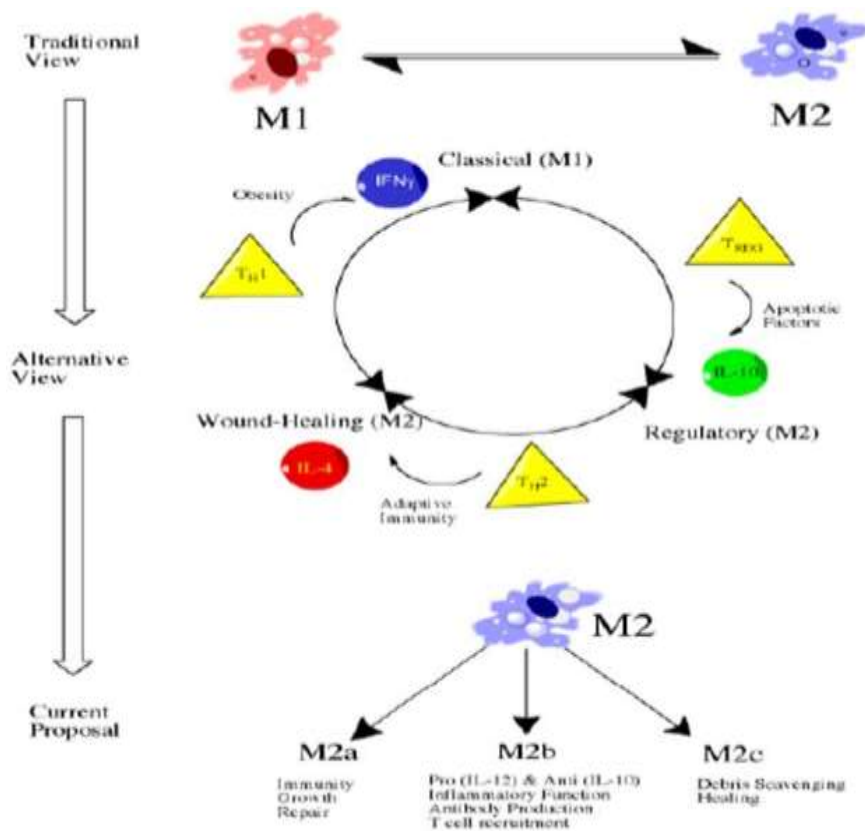


Figure 4.4. *Macrophage polarization*: Classical M1 vs Alternative M2: Evolution of views of macrophage polarization from the more simplistic M1 or M2 characterization without subtypes to the alternative or developing view with the M2 population divided into “wound-healing” and “regulatory” subtypes. Even more current proposals identify three populations of M2-related macrophages involved in a range of functions.

ALTERNATIVELY ACTIVATED MACROPHAGES (M2)

Alternative macrophage activation, similar to classical macrophage activation, can occur in response to both innate and adaptive signals (Mosser D. M. 2007). The primary signaling molecule responsible for activation is interleukin-4 (IL-4), a cytokine released upon initial tissue injury as well as upon initiation of the adaptive immune response.

Alternatively activated macrophages are largely described as beneficial, particularly when it comes to wound healing. Specifically, alternative macrophage activation is responsible for production of extracellular matrix, a property essential in the reparative response. Alternatively activated macrophages are also associated with a reduced expression of pro-inflammatory cytokines, namely TNF α , IL-1b, IL-2, IL-8, IL-12, and CXCL10 (David S. 2011). The response of IL-4 stimulated alternatively activated macrophages is much more characteristic of a TH2-mediated response in comparison to the classical macrophage activation which more closely parallels that of a TH1 response. Despite the previous

characterization of alternatively activated macrophages as a distinct cellular population, emerging evidence indicates the presence of further subtypes under the M2 umbrella and that they are involved in a variety of functions ranging from wound-healing to regulatory functions. In fact, some have described M2 macrophages as actually three distinct subtypes—M2a, M2b, and M2c, each of which is generated through a unique polarization process and performs distinct functions (Figure 4.4) (David S. 2011). M2a and M2c have been attributed clear anti-inflammatory and reparative roles, whereas M2b are somewhat unique in that they produce high amounts of IL-10, an anti-inflammatory cytokine, but low-levels of IL-12, a pro-inflammatory cytokine. Similarly, M2b macrophages may produce TNF α , IL-1 β , and IL-6, indicating a more complex role in the inflammatory response than previously recognized (David S. 2011).

NEURONAL RESCUE

Another critical issue in stroke therapy is how to recover areas of infarcted brain that appear irreversibly damaged due to ischemia. To address this issue, research is now focused on understanding neurogenesis, particularly endogenous repair mechanisms such as the involvement of neuronal progenitor cells and their proliferation and differentiation into neural cells (Wiltrout C. 2007). However, use of a small population of endogenous neuronal progenitor cells and their limited capacity to regenerate under inflammatory conditions may not be enough to achieve significant neurological recovery. Therefore, several growth factors (e.g., epidermal, fibroblast, and vascular endothelial growth factors, erythropoietin) have been tested to increase proliferation and migration of progenitor cells. The major challenge seems to be inducing sufficient proliferation to repair the devastating neuronal damage following ischemia, promoting survival of the newly formed cells in the hostile environment, and more importantly, inducing proper connectivity of the newly formed cells with the existing circuitry (Wiltrout C. 2007). An alternative approach is the transplantation of stem cells, but a number of factors could influence the success of this approach, the main one being the survival of the transplanted cells and the likelihood of their proper differentiation into neuronal cells (Kennea N. L. 2002, Roitberg B. 2004).

FUTURE DIRECTIONS: THERAPEUTIC TARGETING OF MACROPHAGE SUBSETS

Therapeutically, it is clear that shifting from the pro-inflammatory macrophage phenotype, M1, to the anti-inflammatory phenotype, M2, may confer an advantage in both initial injury and long-term recovery. How precisely to alter the phenotype and at what time post-injury is not clear but the idea is promising, particularly considering that recent studies have demonstrated that macrophage subsets may not be truly distinct entities and rather may undergo switching. This idea has been demonstrated *in vitro* in which activating macrophages with LPS resulted in diminished reactivity to subsequent pro-inflammatory stimuli (Lawerence 2011). In contrast, these macrophages maintained the ability to express other genes associated with anti-inflammatory activities such as IL-10. The ability to diminish

responsiveness to pro-inflammatory stimuli while maintaining the anti-inflammatory capabilities is referred to as endotoxin tolerance and is generally associated with a switch from the M1 to M2 phenotype (Lawrence 2011). While inflammation is clearly a promising therapeutic target, our understanding of inflammatory processes and when precisely each event occurs remains somewhat unclear and likely varies across individuals. As such, a need exists for not only improved measurement and insight into inflammatory processes but also a personalized approach, a common theme discussed throughout medical research in recent years. One method for potentially achieving both these goals is the use of blood-based or CSF-based biomarkers.

MOVING FORWARD: CHARTING A COURSE TOWARD NEW STROKE THERAPIES

Tremendous progress has been made in the understanding of fundamental mechanisms of neuronal cell death. However, the translation of these powerful molecular and cellular principles into clinically effective neuroprotective therapies in stroke has been challenging. More recently, emerging data from both experimental and clinical studies now suggest that all cell types in the brain participate in the complex pathophysiology of brain injury following stroke. Consequently, therapeutic approaches should target multiple cell types in an attempt to protect their structural and functional integrity and their reciprocal interactions. Protecting the Neurovascular Unit The so-called “neurovascular unit” provides a conceptual framework that integrates responses in all cell types, including neuronal, glial, and vascular elements (del Zoppo, 2009; Iadecola, 2004; Zaccagna et al., 2008) (Figure 4.5). Cell-cell interactions in the neurovascular unit form the basis of brain function. Dysfunctional signaling in the neurovascular unit underlies the basis for disease. Whereas the intricate molecular pathways of cell death in the neuron have been dissected in detail, the mechanisms of how the entire neurovascular unit responds to stroke are not completely understood. As discussed in the previous sections, cerebral ischemia can rapidly induce perturbations in cell-cell signaling and function within the neurovascular unit. How each cell type succumbs or adapts to injury is dependent on responses in other cells. For example, genomic and proteomic responses in neurons subjected to metabolic stress have been fairly well described. But how each cell type might alter its response to vascular risk factors and ischemia in the context of other elements in the neurovascular unit remains to be elucidated. As the nexus of cell-cell interactions grows, a systems biology approach may eventually be required to rigorously define mechanisms as well as targets for complete neurovascular protection. The importance of cell-cell signaling in the neurovascular unit has been underscored by many studies (Arai and Lo, 2009a, 2009b; Dugas et al., 2008; Eroglu et al., 2009; Guo et al., 2008). Hence, under normal conditions, trophic coupling within the neurovascular unit helps maintain homeostasis. In contrast, under diseased conditions, it is likely that loss of coupling would lead to the pathophysiology (Grammas et al., 1999; Nagai et al., 2007). Loss of trophic coupling is also likely to play a role in the way vascular risk factors render neurons and glia more vulnerable, such as in the link between leukoencephalopathy and brain dysfunction underlying cognitive impairment. Recently, BDNF has been implicated as a candidate factor that mediates trophic coupling between vascular and neuronal compartments (Arai and Lo, 2009b; Guo et al., 2008). So far as BDNF polymorphisms have been implicated in modulating synaptic

plasticity (Kleim et al., 2006), these neurovascular trophic signals may also influence the mechanisms of recovery after stroke.

The concept of the neurovascular unit may provide an integrated framework for investigating mechanisms and therapies. Salvaging neurons alone may not be sufficient. One has to protect glial and vascular elements as well. Future investigations might be better served by pursuing targets that are expressed on multiple cell types in brain. Furthermore, prevention of cell death alone may also not be sufficient. In order for therapies to be clinically meaningful, cellular function must be preserved and/or restored. Neurons that are alive but lack proper connectivity or do not express the correct array of transmitter release-reuptake and synaptic activity may not be functional.

Astrocytes that survive, but cannot provide hemodynamic coupling would be unable to link neuronal activation with the required vascular responses. Oligodendrocytes that are respiring but metabolically impaired might not provide the necessary myelination for axonal conduction. Endothelial cells that survive but do not maintain blood-brain barrier properties would facilitate damage to adjacent parenchyma. Ultimately, any stroke therapy must include both prevention of cell death as well as rescue of integrated neurovascular function. Ischemic tolerance and postischemic hypothermia are some of the most potent protective strategies for ischemic brain injury and represent examples of multimodal therapies targeting the neurovascular unit as a whole. These are described in the next sections.

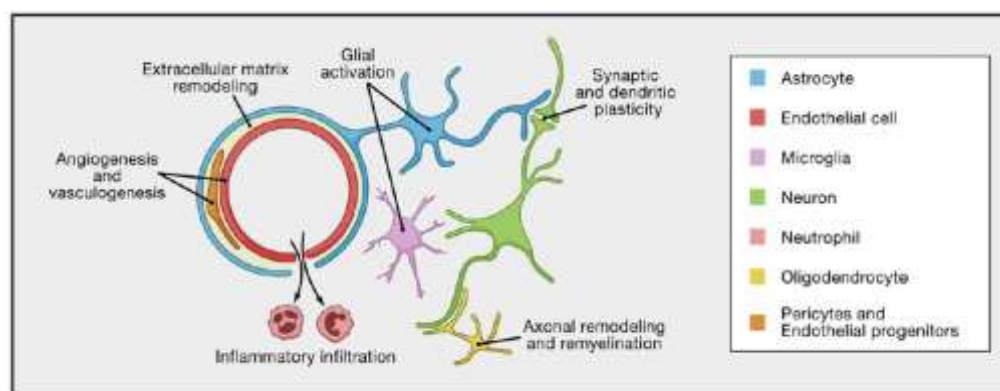


Figure 4.5. *Remodeling the Neurovascular Unit*: This schematic depicts a cerebral blood vessel and surrounding brain parenchyma that comprise the neurovascular unit. The neurovascular unit encompasses cell-cell and cell-matrix interactions between component vascular, glial, and neuronal cells. Homeostatic signaling in the neurovascular unit sustains normal brain function. Dysfunctional neurovascular signaling mediates injury after stroke. During the recovery phase after stroke, neurovascular signaling may also be critically important with repair mechanisms that may involve angiogenesis, vasculogenesis, and endothelial precursor mechanisms. As the blood-brain barrier heals, inflammation may convert from a deleterious to beneficial role. Reactive glia (astrocytes, microglia, pericytes) may secrete trophic factors. Synaptic and dendritic plasticity as well as axonal remyelination may provide parenchymal substrates for functional recovery. Emerging data now suggest that these phenomenon do not occur in isolation. All components of the neurovascular unit are likely to remodel coordinately (Arai et al., 2009; Murphy and Corbett, 2009; Zhang and Chopp, 2009).

HOW DOES THE BRAIN PROTECT ITSELF? LESSONS FROM ISCHEMIC TOLERANCE

Some of the very same mechanisms implicated in the tissue damaging responses after stroke can also be engaged to protect the brain after threatened injury and to induce ischemic tolerance (IT). IT is a concerted organ response to protect itself against tissue injury and has been widely studied over the past three decades in an effort to identify endogenous mechanisms of protection and to exploit them for therapeutic purposes (Obrenovitch, 2008). In its delayed form, it is induced after challenge by a subthreshold noxious stimulus, developing within 1–3 days and lasting for several additional days. Experimentally it can be induced by exposure to preconditioning stimuli such as neurotoxins (e.g., glutamate), substrate deprivation (e.g., ischemia, hypoxia), or inflammation (e.g., cytokines and immune responses) and engages mechanisms that detect, transduce, and promote a complex tissue reaction that reflects a fundamental genomic reprogramming after threatened injury (Dirnagl et al., 2009; Gidday, 2006; Stenzel-Poore et al., 2004). In this altered physiological state, tissue damage is mitigated when challenged by exposure to a wide range of deleterious stimuli, even if unrelated to the initial challenge. It is mediated by highly redundant cellular, molecular, and physiological mechanisms involving neurons, astrocytes, and vascular cells (Gidday, 2006). Upstream sensors have been implicated, including adenosine and its A1 receptor, the NR2A NMDA receptor subunit, and the transcriptional regulator hypoxia inducible factor (HIF). HIF is an attractive candidate because it regulates a constellation of genes, including those whose protein products facilitate oxygen transport (erythropoietin), regulate glycolytic metabolism (e.g., glucose transporters), and promote angiogenesis (e.g., VEGF), by way of example.

Unexpectedly, genetic deletion of HIF1a improves infarct volume after middle cerebral artery occlusion, implicating as yet unknown pathogenic mechanisms activated by ischemia (Helton et al., 2005). Erythropoietin, one of the gene products of HIF1a, promotes PI3 kinase/AKT activity and the expression of NFkB-dependent protective genes (Digicaylioglu and Lipton, 2001).

Erythropoietin also downregulates hypoxia-induced proapoptotic mechanisms (Noguchi et al., 2007; Prass et al., 2003; Ruscher et al., 2002), and erythropoietin-based therapies have been investigated in the clinical arena (Ehrenreich et al., 2009). Heat shock protein family members are upregulated in ischemic tolerance and, based on their multiple cytoprotective actions, may promote survival. Preconditioning stimuli preserve connexin hemichannels in astrocytes facilitating ATP released from these cells and increasing the extracellular concentration of the beneficial nucleoside adenosine (Lin et al., 2008).

At the same time, preconditioning safeguards the function of cerebral blood vessels from the deleterious effects of cerebral ischemia, resulting in improved penumbral flow and reduced brain damage (Kunz et al., 2007b). These astrocytic and vascular changes are coupled with mechanisms that reduce energy demands, lessen inflammatory responses and ion channel activity, and decrease cascades promoting blood clotting and apoptotic cell death (Dirnagl et al., 2009; Obrenovitch, 2008; Gidday, 2006). Factors involving mitochondria as well as its uncoupling proteins have also been suggested and include improved efficiency of ATP synthesis, preservation of the mitochondrial membrane potential, and delayed opening of mitochondrial transition pore formation, as well as opening of mitochondrial ATP-

dependent K⁺ channels (Dirnagl and Meisel, 2008). One of the key questions defying explanation at the moment is how and why diverse preconditioning stimuli targeting single genes or key steps in seemingly unrelated cascades all impact infarct injury in genetically engineered and wild-type mice. It may be telling us that the mechanisms through which the brain protects itself involve a highly integrated network response that emphasizes multiple cells within the neurovascular unit. If true, comparable multicellular approaches are needed that capitalize on this integrated response in the design of successful treatments.

THERAPEUTIC HYPOTHERMIA: ENGAGING PLEIOTROPIC MECHANISMS IN MULTIPLE CELL TYPES

The therapeutic hypothermia field has advanced significantly during the past decade, inspired by positive outcomes from clinical trials in cardiac arrest victims (Bernard et al., 2002). Satisfactory neurological recovery was achieved in about half of the treated patients when treatment was begun within 2 hr of cardiac arrest. Promising results were reported in small feasibility studies in neonatal ischemia (Shankaran et al., 2005). Current efforts are directed toward translating these successes in cardiac arrest and neonatal ischemia to patients with ischemic stroke, but there remain unresolved technical issues surrounding the timing and method for inducing hypothermia, and the deleterious effect of rewarming (Wagner and Zuccarello, 2005). It is now well accepted that hypothermia reduces ischemia in animal models of stroke, and so far, preclinical results have helped guide the design of clinical trials in human subjects. Combining hypothermia with thrombolysis is one example. Experimental evidence suggests that hypothermia protects the brain through pleiotropic effects, acting on multiple cell types within the neurovascular unit. Hypothermia reduces brain metabolism, thereby significantly decreasing oxygen consumption and the workload of the entire organ (Sakoh and Gjedde, 2003). Hypothermia also reduces glutamate release, ROS production, and peri-infarct depolarizations. Inflammatory markers decrease, as well as microglial activation, leucocyte infiltration, MMP activation, and BBB disruption. In addition, hypothermia suppresses apoptotic cell death, decreases mitochondrial release of cytochrome C and apoptosis inducing factor (see for review Tang and Yenari, 2010). Cell survival pathways are upregulated and appear to contribute to the multiplicity of neuroprotective mechanisms. To what extent each of these promotes tissue protection or interacts synergistically to enhance tissue survival is not known and remains for further investigation. Nevertheless, therapeutic hypothermia does illustrate the potential power of approaching stroke therapy using combinations of modalities that engage multiple cell types to enhance protection and recovery.

GAINING INSIGHT FROM THE BEDSIDE: UTILITY OF BIOMARKERS

Biomarkers for Diagnosis and Prognosis

With the recent advances in technology and research, many look for biomarkers as a new avenue to further understand, diagnose, and treat diseases. A biomarker is a biological

component that can be tangibly quantified to understand normal or pathological functioning in the body (Figure 2) (Bettermann K. 2011, Mayeux R. 2004). The roles of biomarkers in the laboratory and clinical setting are vast. They can be used in diagnosing pathologies, monitoring progress of disease or injury, identifying targets for intervention, and assessing risk of pathology. The measurement of biomarkers translated successfully from bench-to-bedside with troponin-1, a well-known example of a biomarker used to aid in diagnosis of myocardial infarction (Than M. 2012). Within the neuroscience community, biomarker research has become an area of increased interest and investigation for a variety of pathologies ranging from stroke to traumatic brain injury (TBI) to Alzheimer's disease (AD).

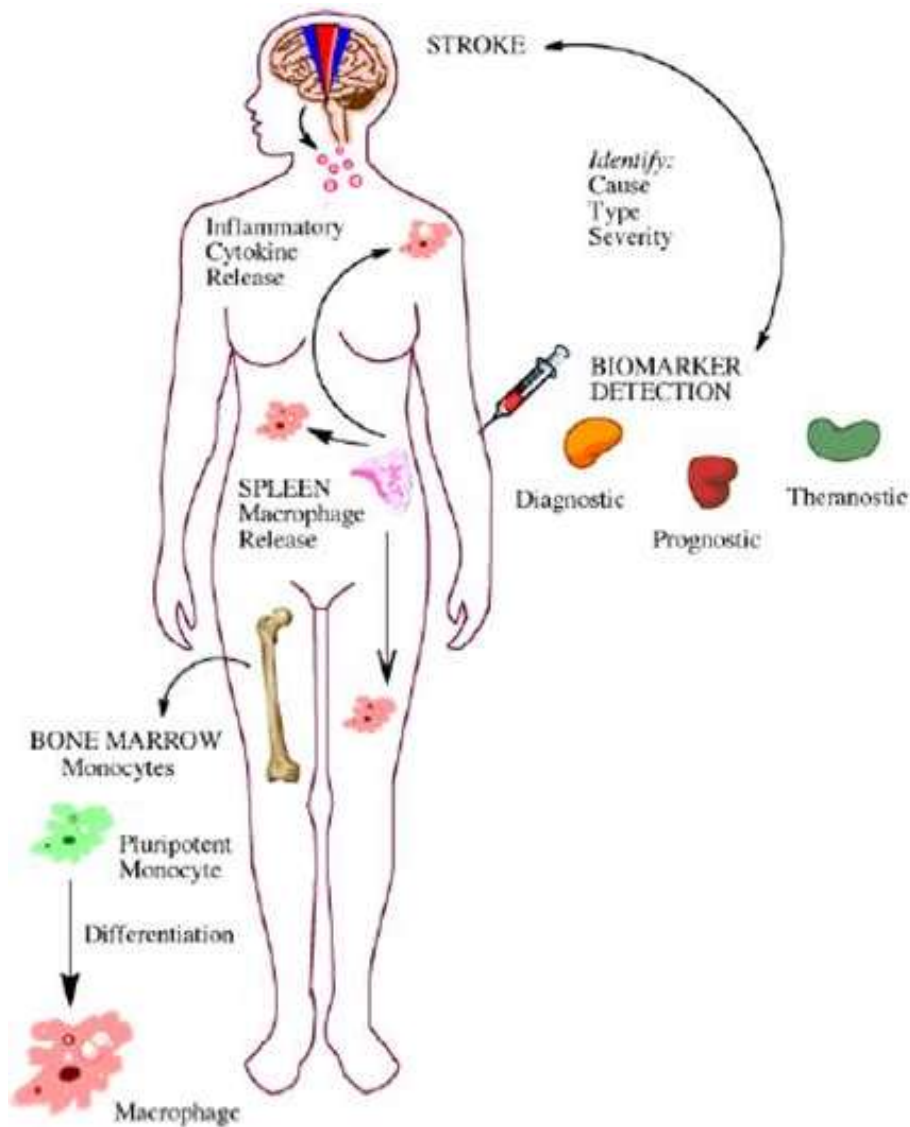


Figure 4.6. *Potential uses of biomarker in Stroke*: Potential uses of biomarkers include identification of stroke cause, type, and severity allowing for potential diagnosis, prognostication, and therapeutic selection or monitoring (theranosis). Biomarkers may also play a role in understanding disease pathophysiology, particularly in regards to inflammation and macrophage release and/or polarization.

The remainder of our discussion of biomarkers will focus on ischemic stroke, but it is important to recognize the similarities of many disease states, particularly stroke and TBI. Timing of an accurate diagnosis is essential for patients with stroke. The only FDA-approved treatment, tissue plasminogen activator (tPA), has traditionally had a 3-h window for administration after onset of stroke (Weant K. A. 2012). While recent guidelines suggest up to a 4.5 h window (Lansberg M. G. 2012), tPA administration has the potential to result in numerous well-documented adverse effects, such as hemorrhagic transformation (HT) or even death (Montaner J. 2011). Consequently, numerous contraindications for tPA administration exist and many patients go untreated. The identification of biomarkers for stroke therefore may not to be limited to traditional diagnostic or prognostic avenues but also more appropriate identification of patients at risk for tPA-associated morbidities.

Likewise, biomarkers may also be useful in determining patients in which tPA administration may be beneficial despite the presence of a comorbidity or risk-factor that would normally result in exclusion from tPA-based treatment. Previous studies have demonstrated differences in numerous markers that may one day potentially play a role in detection of ischemic stroke, stroke-mimic, and transient attack, as well as predict patient outcome (Navaro Sobrino M. 2011, Quin L. 2012), determine stroke severity, predict the risk of recurrent stroke (Tsai T. H. 2012) or hemorrhagic transformation (HT) (Ramos-Fernandez M. 2011) increase understanding of pathophysiology behind cerebral ischemia (Vangilder R. L. 2012), and provide possible targets for therapeutic intervention (Denes A. 2011). While additional studies are certainly required to elucidate the most appropriate use of the markers discussed in the cited studies, each of these studies has provided valuable insight and potential examples of biomarker utilization at various time-points in the therapeutic or prognostic decision-making process, particularly as related to patient suitability for tPA administration. Historically, biomarker-based studies sought the identification of a single marker for diagnostic/prognostic purposes. However, the investigated biomarkers have proven to be not specific or sensitive enough across multiple populations for clinical usage. Thus, researchers have transitioned largely towards identification of a panel of biomarkers rather than a single marker. The discovery of a group of biomarkers, instead of a sole marker, may improve the specificity, sensitivity, and prediction values across time (Ramos-Fernandez M. 2011, Bettermann K. 2011). A panel could therefore additionally provide the healthcare provider with more information than a single biomarker could reliably do alone (Ramos-Fernandez M. 2011). Due to biomarker discovery and investigation being a relatively new field, our discussion below is limited primarily to single marker studies.

Multiple biomarkers of stroke have been identified in the literature. A popular biomarker of note is matrix metalloproteinase 9 (MMP-9). High levels are correlated with current neurological deterioration, stroke severity, and infarct size (Montaner J. 2001). Interestingly, before administration of tPA, high levels also have been shown to accurately predict HT (Castellanos M. 2003, Montaner J. 2003). Despite MMP-9 playing a clear role in stroke pathology, a major pitfall of its use in the clinical setting is that it is not specific to the brain. It is involved with inflammation and matrix remodeling throughout the body and in different pathologies such as diabetes, hypertension (Tayebjee M. H. 2004), atherosclerosis (Sundstrom J. 2006), and many others. Therefore, MMP-9 likely cannot be used as a stand-alone biomarker for stroke; however it may be a helpful addition to a biomarker panel. Another biomarker of note is glial fibrillary acidic protein (GFAP). High levels of GFAP have been found to be indicative of hemorrhagic stroke in the acute stage, and therefore,

GFAP may be an important biomarker to consider when deciding the appropriateness of thrombolytic administration (Hasan N. 2012). Importantly unlike MMP-9, GFAP is released strictly by astrocytes in the brain (Marginean I. C. 2011). Outside of stroke, GFAP possesses prognostic value for outcome in cases of mild and severe traumatic brain injuries, though it is suggested that it is not an independent factor (Metting Z. 2012, Wiesmann M. 2010). A high concentration of S100 calcium binding protein (S100B) also has the ability to distinguish between hemorrhagic and ischemic stroke (Montaner J. 2012) and positively correlates with infarct volume (Jonsson H. 2001). When measured along with MMP-9, Glickman and colleagues revealed that S100B levels can differentiate between ischemic stroke and stroke mimic (Glickman S. W. 2011). Yet also like MMP-9, evidence indicates that S100B is expressed outside of the brain, such as in cancer, and therefore suffers from the same lack of specificity associated with other markers previously mentioned (Peric B. 2011, Strobel K. 2007). D-dimer concentration shows promise as a biomarker of stroke. As a component of a blood clot, d-dimer is useful for diagnosing the subtype (Hirano K. 2012), specifically cardioembolic stroke (Alvarez-Perez F. J. 2011), and distinguishing between ischemic stroke and stroke-mimic (Montaner J. 2011). As a thrombotic biomarker, d-dimer has many uses in regards to stroke, but it too is found throughout the body and, therefore, is not reflective solely of central nervous system events. Regardless, d-dimer has proven useful in the diagnosis of post-stroke cardiovascular complications such as pulmonary embolism, deep vein thrombosis and others (Glickman S. W. 2011).

The idea of individualized therapy, particularly when it concerns the selection of patients for thrombolytic therapy, has been further explored in recent years by Ning and colleagues. They showed significant prolonged effects on cellular signaling following tPA as evident by prolonged patterns of degradomic protein expression up to 5 days post-treatment, a finding in distinct contrast to that of non-tPA treated stroke patients in which a hyperacute response was evident but ceased by 24 h (Ning M. 2010). Besides demonstrating widespread effects of tPA on the extracellular matrix and brain parenchyma, far outside of the acute therapeutic window, their work could potentially demonstrate the utility of biomarkers in identification of patients at risk for adverse effects following therapeutic administration.

Biomarkers for Elucidating Disease Pathophysiology

While biomarkers are often thought of primarily for diagnostic and prognostic purposes, emerging work indicates a potentially greater role for biomarkers in elucidation of disease pathophysiology, especially communication between the brain and systemic responses. For example, Hayakawa and colleagues showed that by disrupting either the release of high-mobility-group-box-1 (HMGB1) from reactive astrocytes, or the HMGB1 receptor on endothelial progenitor cells, neurovascular remodeling is altered, and worsened deficits result (Hayakawa K. 2012). This work demonstrates the ability to isolate cell-specific events by taking in vitro based findings, such as the release of HMGB1 into astrocyte-conditioned media, and applying them to in vivo studies to determine the effect of HMGB1 systemically on post-ischemic injury. Other studies have used in vitro models in a similar fashion to demonstrate complex signaling relationships amongst neurons and glia. Specifically, Chu et al. showed the protective role of factors released by ischemic microglia, astrocytes, and neurons on astrocytes subjected to ischemia (Chu L. F. 2008). While space constraints

prevent the mention of all applicable works, studies such as those described illustrate the potential for an expanded role of biomarkers in elucidation of disease pathophysiology and crosstalk between various cells within the brain and systemically.

KEY ADVANCES IN STROKE

■ ■ ST-3 reinforces the need for further efforts to increase the proportion of ischaemic strokes treated within 3 h, but provides reassurance that thrombolysis in elderly patients or within 6 h from stroke onset does not increase mortality (Sandercock, P. 2012)

■ ■ Under the conditions of the ICTUS trial, citicoline is not effective in the treatment of moderate to severe acute ischaemic stroke (Dávalos, A. 2012)

■ ■ Atrial tachyarrhythmia must be considered as a new source of embolism in patients with cryptogenic stroke (Healey, J. S 2012)

■ ■ Patent foramen ovale closure with a device did not offer a greater benefit than medical therapy alone for the prevention of recurrent stroke or TIA (Takahashi, K. 2006)

■ ■ An animal study provides the first evidence that transplantation of cells derived from human induced pluripotent stem cells is a safe and efficient approach to promote recovery after stroke (Oki, K. 2012)

Several clinical trials and experimental studies that could have a major impact on the treatment of patients with ischaemic stroke were published in 2012. The studies cover all therapeutic options, including stroke prevention, recanalization and thrombolysis, neuroprotection, and promising new therapeutic approaches focused on neurorepair. Despite improvements in its management in recent years, cerebrovascular disease remains a major cause of mortality and morbidity. The current framework of therapeutic options to prevent or treat stroke comprises a spectrum of five fields of treatment—primary prevention, recanalization and thrombolysis, neuroprotection, secondary prevention, and neurorepair (Figure 5.6). In 2012, we witnessed developments in all five areas, with important implications for stroke treatment. When primary prevention fails, an effective protocol for acute stroke treatment is imperative, early reperfusion of ischemic tissue being the primary goal. Currently, thrombolytic therapy with recombinant tissue plasminogen activator (rtPA) is the most effective approach. However, the extensive exclusion criteria, together with the short therapeutic window and narrow age range, severely restrict its use. The Third International Stroke Trial (IST-3) (Sandercock, P. 2012) was conducted to establish the balance between benefits and harms of rtPA treatment in patients who did not accomplish the licence criteria and, hence, to determine whether a wider range of patients might benefit up from rtPA up to 6 h from stroke onset. IST-3 enrolled 3,035 patients (1,515 in the rtPA group and 1,520 controls), 53% of whom were aged >80 years. No differences were found between groups with respect to the primary end point (37% in the rtPA group versus 35% of controls survived and achieved independence).

The global benefits of rtPA treatment seemed to be greatest within the first 3 h from stroke onset, but the analyses had insufficient power to define the benefit in a therapeutic window beyond 3 h. Interestingly, treatment efficacy was similar between patients older and younger than 80 years.

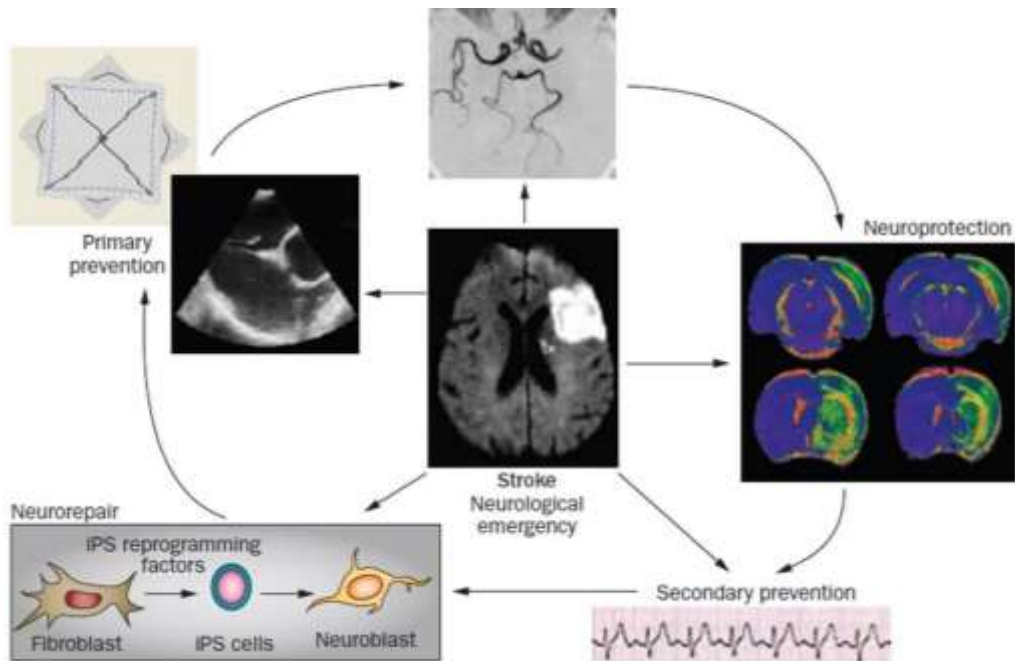


Figure 5.6. *Recent advances in stroke*: Spectrum of therapeutic options to prevent or treat stroke. In 2012, key advances were made in stroke prevention (primary and secondary), recanalization and thrombolysis, neuroprotection, and neurorepair strategies. Abbreviation: iPS, induced pluripotent stem.

The IST-3 data reinforce the need for further efforts to increase the proportion of ischemic strokes treated within 3 h, but they provide reassurance that rtPA treatment in elderly patients and within 6 h from stroke onset does not increase mortality. Although clinical trials with neuroprotective agents have systematically failed, neuroprotection remains a treatment option for acute ischemic stroke. In 2012, the results of ICTUS (International Citicoline Trial on acUte Stroke) were published (Dávalos, A. 2012). In this trial, patients with moderate to severe acute ischemic stroke were treated with either citicoline or placebo within 24 h after symptom onset. The primary outcome was recovery at 90 days measured by a global test combining three measures—NIH Stroke Scale score ≤ 1 , Modified Rankin Scale score ≤ 1 , and Barthel Index score ≥ 95 —in accordance with a pooled meta-analysis of previous randomized trials with citicoline.

(Dávalos, A. 2002) 2,298 patients (1,148 assigned to citicoline and 1,150 to placebo) were enrolled in ICTUS.2 Global recovery was similar in both groups, and no significant differences were reported in the safety variables or the rate of adverse events. Although ICTUS followed a protocol nearly identical to that of the pooled meta-analysis, (Dávalos, A. 2002) with minimal differences in statistical analysis, none of the benefits for citicoline were confirmed. However, the previous clinical trials with citicoline were conducted 10 years ago, and the standard of stroke care has substantially improved in the meantime. Also, the patients in ICTUS were, on average, 4 years older, and were over three times more likely to have received rtPA treatment, than those in previous trials. Comparison with the older studies suggests that the benefits of citicoline have become diluted in parallel with improvements in the standard of care for acute ischaemic stroke—a fact that should be taken into account for future trials of neuroprotective drugs.

To initiate appropriate secondary prevention, it is essential to know the cause of stroke, yet at least one-quarter of strokes are still classified as cryptogenic. The ASSERT investigators recruited 2,580 individuals >60 years without a history of atrial fibrillation (AF) in whom a pacemaker or defibrillator had been implanted. (Healey, J. S 2012) Patients were monitored for 3 months to detect subclinical atrial tachyarrhythmias (ATs; episodes of atrial rate >190 bpm for >6 min), and were followed up for a mean of 2.5 years for the primary outcome of ischemic stroke or systemic embolism. At least one AT was detected in 10.1% of patients. During the follow-up period, 4.2% of patients in whom subclinical AT had been detected had an ischemic stroke or systemic embolism, compared with 1.7% in whom subclinical AT was not detected. The attributable risk of ischemic stroke or systemic embolism associated with subclinical AT was 13%, which is similar to the stroke risk associated with AF (Wolf, P. A. 1991) On the basis of these results, AT must be considered as a new source of embolism in patients with cryptogenic stroke.

The prevalence of patent foramen ovale (PFO) ranges from 20–26% in the general population, but may be as high as 56% in patients under 55 years who have experienced a cryptogenic stroke. (Lamy, C. 2002) The CLOSURE I investigators evaluated the potential benefits of percutaneous device closure versus medical therapy for secondary stroke prevention in patients with PFO (Furlan, A. 2012) This multicentre, randomized, open-label trial compared PFO closure (using the STARFlex device) with medical therapy (warfarin, aspirin or both) in patients 18–60 years of age with PFO who had presented with cryptogenic stroke or TIA within the previous 6 months.

The primary end point was a composite of stroke or TIA during 2 years of follow-up, death from any cause during the first 30 days, or death from neurological causes between 31 days and 2 years. 909 patients—447 percutaneous device and 462 medical therapies—were enrolled. At 2 years, effective closure was maintained at 86.7% of cases. The incidence of the primary end point was 5.5% in the percutaneous device group and 6.8% in the medical group. Stroke incidence was 2.9% in the percutaneous device group and 3.1% in the medical group. No deaths occurred by 30 days in either group, and there were no deaths from neurological causes during the 2-year follow-up period. AF was significantly more frequent in the closure group (5.7% versus 0.7%). In conclusion, closure did not offer greater benefits than medical therapy alone for the prevention of recurrent stroke or TIA. Over the past two decades, stem cell based neurorepair has emerged as a promising therapeutic option for ischemic stroke.

Animal experimental data with embryonic stem (ES) cells, neural progenitor cells or bone marrow-derived progenitor cells are encouraging; however, no proven stem cell-based therapy is currently available for stroke. Despite the supposed immune-privileged status of the CNS, allogeneic grafts of stem cell-derived neurons and glia remain susceptible to rejection.

A novel alternative strategy to avoid graft rejection and immunosuppression is to generate induced pluripotent stem cells (iPSCs) from somatic cells. In 2006, it was reported that skin fibroblasts from adult mice could be reprogrammed to a pluripotent state by retroviral expression of four transcription factors (Takahashi, K. 2006). The resulting iPSCs are indistinguishable from ES cells in morphology, proliferative capacities, surface antigens, gene expression, epigenetic status, and telomerase activity. The Nobel Prize in Physiology or Medicine 2012 was awarded to Sir John B. Gurdon and Shinya Yamanaka for this breakthrough. The Laboratory of Neural Stem Cell Biology and Therapy, Lund, Sweden

transplanted long-term self-renewing neuroepithelial-like stem cells, generated from adult human fibroblast-derived iPSCs, into stroke-damaged mouse and rat striatum or cortex. (Oki, K. 2012).

The transplanted cells stopped proliferating, could survive without forming tumors for at least 4 months, and differentiated into morphologically mature neurons. Grafted cells exhibited electrophysiological properties of mature neurons and received synaptic input from host neurons. Most importantly, the recovery of forepaw movements was observed by 1 week after transplantation. This study provides the first evidence that transplantation of human iPSC-derived cells is a safe and efficient approach to promote repair and recovery after stroke.

CURRENT DEVICES USED FOR MECHANICAL EMBOLUS REMOVAL IN CEREBRAL ISCHEMIA

The mechanical embolus removal in cerebral ischemia (MERCI) trial evaluated the efficacy and safety of a mechanical endovascular device (which consists of a Nitinol core wire with shaped loops at the distal end designed to engage the thrombus) in the revascularization of an occluded large cerebral vessel within 8 hours of stroke onset (Figure 5.7) (Smith W. 2005 and 2008). The original study was subsequently updated with the Multi-MERCI trial incorporating a newer generation of the device (Smith W. 2008). The population of patients included in both studies were those ineligible to receive IV tPA (either arriving too late or having a contraindication for its use). The MERCI trial was a multicenter, prospective, single-arm trial that referred to the control arm of the PROACT II trial as historical control. Although the therapeutic window was 8 hours, the median time from onset to treatment was 4.3 hours in both studies. The recanalization rate was significantly higher than that in the control arm of PROACT II (48% vs 18%, respectively), but lower than that in the IA tPA group (66%). The proportion of patients achieving mRS2 at 90 days was comparable to that in the control arm of PROACT II (22.6% vs 25%, respectively). In contrast, the mortality rate of 43.5% was significantly higher than that reported in most prospective studies of the treatment of acute stroke. The investigators attributed this elevated mortality to a higher baseline NIHSS score (median 18) and higher proportion of basilar artery and internal carotid artery terminus occlusions. The introduction of a new generation of the device in the Multi-MERCI trial resulted in a significantly higher recanalization rate (55%) and an improvement in the mortality or neurological outcome at 90 days, although statistically insignificant.

PENUMBRA DEVICE

The Penumbra device was introduced in the United States in 2008 after results from a European safety trial were published (Figure 5.8) (Penumbra Pivotal Stroke Group 2009). The system consists of a thrombus debulking and aspiration component as well as a direct thrombus extraction component. The safety and efficacy of the device were reported in a multicenter, prospective, single-arm trial with the goal of establishing substantial equivalence to its predicate device—the Merci clot retrieval system.

Although the revascularization rate using the Penumbra device was the highest of the prospective trials at 81.6% (compared with 55% in the Multi-MERCI trial and 66% in the PROACT II trial), the neurological outcome was comparable or lower (25% with mRS 2, vs 36% in the Multi- MERCI trial and 40% in the PROACT II trial).

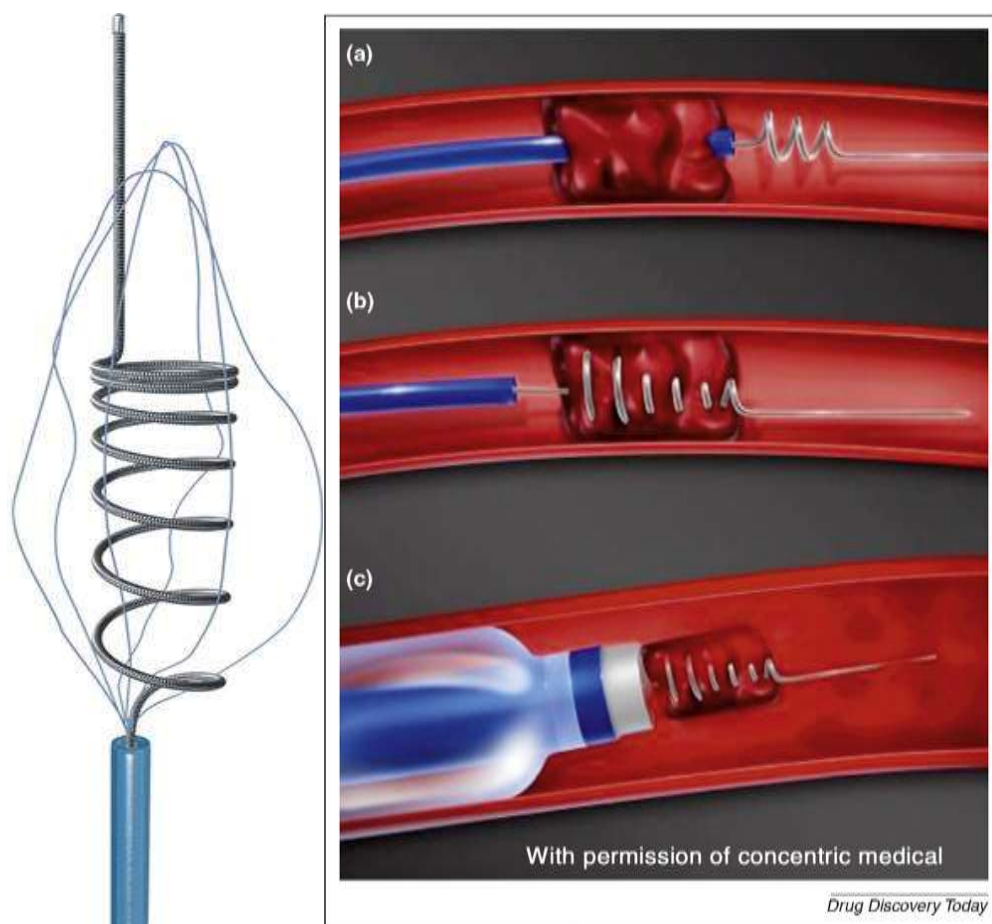


Figure 5.7. (A) *Merci Retriever device* which is a corkscrew-shaped product designed to remove blood clots from large vessels in patients experiencing acute ischemic stroke. (B) *Endovascular Therapy for acute stroke*: The principle of clot retrieving. The Merci System. Endovascular therapy for acute stroke is always performed under general anesthesia. First the MerciBalloon Guide Catheter is placed in the common or internal carotid artery for anterior circulation occlusion. Using standard cerebral catheterization techniques, a microcatheter is guided then through the guide catheter into the occluded vessel and passed beyond the thrombus (a). The Merci Retriever is now advanced through the microcatheter, and several of the helical loops are deployed distal to the thrombus. The Merci Retriever is then retracted to the face of the thrombus, and the proximal loops are deployed within the thrombus (b). The balloon of the Merci Balloon Guide Catheter has to be inflated now to arrest orthograde blood flow during removal of the thrombus. At least the Merci Retriever with the ensnared thrombus and the microcatheter are withdrawn together into the balloon guide catheter lumen. Continuous aspiration is applied to the guide catheter to promote complete evacuation of the thrombus (c). Once a retrieval attempt is completed, the balloon of the balloon guide catheter is deflated to re-establish flow. If the clot could not be removed with the retriever, the procedure can be repeated up to 5 times.

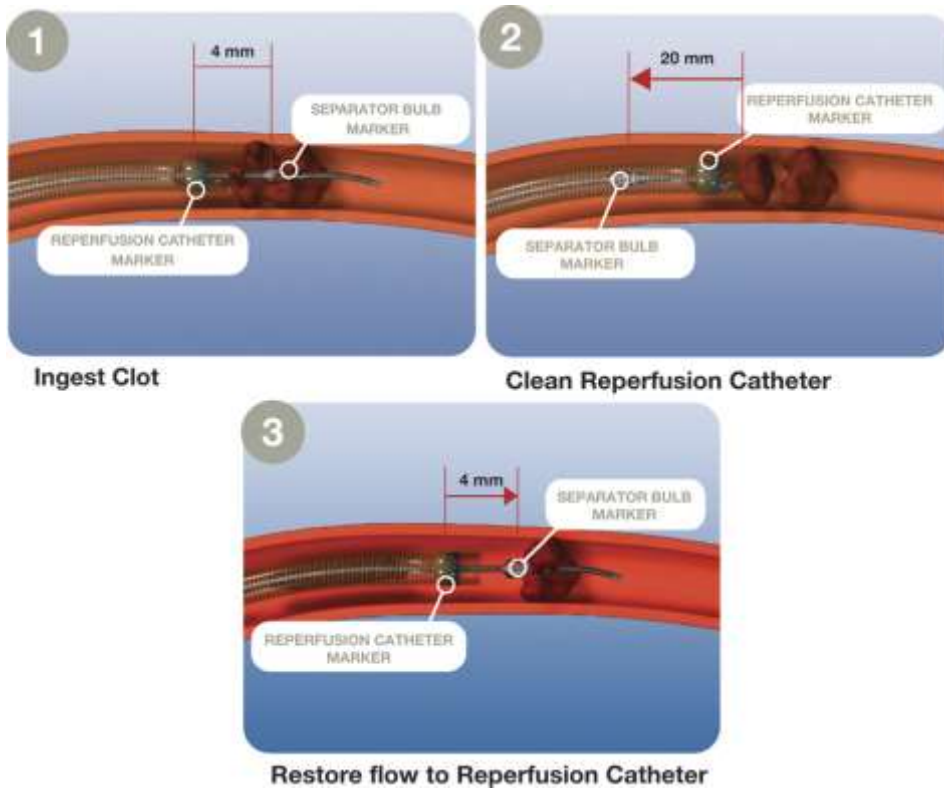


Figure 5.8. Penumbra Device.

The investigators attribute this disparity to the lack of sufficient power and higher baseline NIHSS score.

STENT-ASSISTED RECANALIZATION IN ACUTE ISCHEMIC

Stroke The use of endovascular stents has been recently advocated in the setting of acute stroke (Levy E. 2009). Based on retrospective data regarding the use of intracranial stents as a salvage technique in acute stroke, the SARIS trial (stent-assisted recanalization in acute ischemic stroke) aimed to evaluate the safety of stent deployment as a primary therapeutic intervention for acute stroke using a prospective, single-arm study design.

The investigators used the Wingspan (Boston Scientific, Natick, MA) and the Enterprise (Cordis, Bridgewater, NJ) intracranial self-expanding stents.

All 20 cases included in the trial were successfully revascularized, although 60% of patients required the use of an adjuvant intraprocedural pharmacological infusion (eptifibatide or tPA) or angioplasty. The risk of subsequent asymptomatic and symptomatic hemorrhage was 10% and 5%, respectively, and no procedure-related complications were reported. With regard to neurological outcomes, 45% of patients achieved an mRS score of 1 or less at 1 month follow-up. The results of the SARIS trial are difficult to directly compare with the large randomized prospective trials because it included a small number of patients with a relatively low median NIHSS score (13% vs 19% in IMS II and 17% in PROACT II), was

nonrandomized, and included only short follow-up; nonetheless, the results represent an encouraging foundation for further investigation into the role of stenting for stroke treatment

FUTURE DIRECTIONS IN STROKE THERAPY: LOGISTICS OF CARE

Improved stroke care begins at the pre-hospital stage; The California Acute Stroke Prototype Registry investigators estimate >10-fold increase in the number of patients receiving appropriate treatment if the hospital arrival time is within 1 hour of symptom onset, (California Acute stroke Pilot Registry (CASPR 2005)) highlighting the importance of public education and emergency medical services response time.

Future first responders to the scene of a suspected stroke may be able to perform real-time neuroimaging rapidly with subsequent transmission of images to the receiving medical center such that the time to diagnosis, triage, and catheterization (if deemed necessary) is optimized. Real-time neuroimaging using transcranial ultrasonography, especially with the concurrent use of ultrasonography contrast agents, may prove beneficial in providing such rapid assessment of occluded cerebral vessels in the field (Seidel G. 2009) Color-coded duplex ultrasonography has been shown to correctly differentiate hemorrhagic stroke from ischemic stroke in 95% of cases when compared with computed tomography scanning (Maurer M. 1998). Furthermore, the use of cerebroprotective agents, again possibly administered in the field or early in the provision of stroke care, may further contribute to improved outcomes. With our current knowledge, patients arriving at a stroke treatment center outside of the defined windows for thrombolysis have few options outside of future stroke prevention and rehabilitation; therefore, extension of the therapeutic window is critical for allowing more patients to benefit from acute stroke treatment. The replacement of the traditional “stopwatch” approach to stroke with a more dynamic and functional time window is likely to take place, with growing evidence in clinical trials with the usefulness of magnetic resonance imaging-based patient selection for thrombolysis (desmoteplase in acute ischemic stroke (DIAS), diffusion and perfusion imaging evaluation for understanding stroke evolution (DEFUSE), and dose escalation of desmoteplase in acute stroke (DEDAS) trials) (Goldberg M. 2012). The ongoing Third International Stroke Trial and Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE) trial will also likely impact whether future patients are offered thrombolysis outside the conventional window.

TECHNOLOGICAL ADVANCES

The incorporation of interventional approaches as an adjunct or alternative to intravenous thrombolysis in the setting of acute stroke is a rapidly growing field with a recent explosion in the number of devices available to the interventionalist. The PROACT trial was one of the first large multicenter trials to investigate the use of intravenous thrombolytic agents for the treatment of acute stroke; the study was followed by several other randomized trials, including the IMS study, which described the use of IV tPA as a bridge toward catheter-directed arterial infusion of the thrombolytic. IMS II introduced data regarding the use of low-energy sonography in addition to local thrombolysis. Subsequent studies have evaluated

mechanical clot retrieval devices, including the MERCI and Penumbra trials (Goldberg M. 2012). Investigations and breakthroughs are likely to continue in both pharmacological and mechanical thrombolysis, with the future generation of devices likely to include a combination of the 2 modalities to achieve increased recanalization rates (Hopkins L. 2008, Sugrue P. A. 2009). Future devices are also likely to address the issue of distal emboli during clot manipulation (Leibig T. 2008). In addition to clot removal and microinfusion devices, the use of retrievable stents for the acute revascularization of thromboembolic stroke is a topic of great interest that is currently being evaluated (Wakhloo A. 2008, Zaidat O. O. 2008). Stenting may prove useful as an adjunct to thrombolysis for immediate reperfusion with subsequent retrieval, or alternatively left in place for permanent deployment.

PHARMACOLOGICAL ADVANCES

Promising pharmacological options for the treatment of acute ischemic stroke can be organized in 1 of 3 categories:

1. Novel thromboembolic agents and adjuvants (including antiplatelet and anticoagulation agents). Microplasmin, a recombinant form of human plasmin, may have a reduced propensity to cause bleeding compared with tPA and has been shown to be tolerable in human volunteers and efficacious in animal models of stroke. Microplasmin is currently under investigation to determine the clinical efficacy in human acute ischemic stroke patients (Thijis V. 2009).
2. Neuroprotective agents that have been an attractive but so far elusive target. At the time of this writing, more than 160 trials for ischemic stroke neuroprotective agents have been initiated (Internet Stroke Centre W. U. 2010). Unfortunately, few have produced positive results. However, notable recent exceptions include high-dose human albumin therapy, which showed promising results in the original Albumin in Acute Stroke trial (Palesch Y. Y. 2006). A larger randomized multicenter placebo-controlled efficacy trial is now underway (Goldberg M. 2012). Additionally, magnesium, which has been shown so far to be a rather weak neuroprotectant, (Muir K. W. 2004) continues to be under investigation because it is a safe and relatively inexpensive agent that may be administered in the hyperacute pre-hospital setting or during interventional procedures (Goldberg M. 2012)
3. Agents that contribute to the recovery of lost or dormant neuronal function. Although currently at its infancy stages, future interventional approaches may include local administration of neurorestorative agents, such as stem cells of both exogenous and endogenous origins, or neurotrophic molecules that promote and direct endogenous neurogenesis (Burns T. C. 2009, Chen J. 2001).

CONCLUSION

The lack of successful therapeutic translation from bench-to-bedside for a devastating disease such as stroke, despite the progression of over 100 proposed therapeutics to clinical

trials, necessitates a closer review of both how stroke research is conducted as well as the therapeutic targets selected. While preclinical studies have improved in rigor, particularly with the advent of the Stroke Therapy Academic and Industry Roundtable (STAIR), clear deficiencies remain. Perhaps most notably, the lack of emphasis on the aging process and commonly associated comorbidities found in stroke patients, is persistent throughout the literature. As aging is the greatest risk factor for stroke, and numerous works have identified a role of aging in response to injury, inclusion of more preclinical models utilizing aged animals may lead to the development of promising therapeutics. Other promising avenues of investigation into stroke research include the role of glia, particularly with regards to the inflammatory response.

Stroke remains a major public health issue, and thrombolysis have been proven effective as therapy for cerebral ischemia, either administered intravenously within 3 hours or intra-arterially within 3 to 6 hours. Whereas the development of new thrombolytic agents and regimens as well as the rapid evolvement of endovascular therapies are an exciting component of the future of stroke treatments, one of the main challenges remains the wide availability and use of treatment for acute stroke patients. Even in its most basic version, intravenous thrombolysis has been demonstrated to be effective and safe when used in small medical centers. The advent of telemedicine and the use of robotics play an important role in making the therapy available to all stroke patients, as does community education, training of primary care providers, allied health professionals, and emergency medical services.

A number of promising thrombolytic strategies are currently being investigated, which may push the field further forward, including the following: The pilot nonrandomized safety and feasibility study, Interventional Management of Stroke, analyzed a combination of intravenous and intra-arterial thrombolysis, bringing together the speed of initiation of intravenous therapy and the higher recanalization rates of intraarterial thrombolysis. Endovascular therapies are rapidly evolving and continue to offer patients in whom intravenous thrombolysis fails a real chance at recanalization. These developments are of great importance to the endovascular neurosurgeon, and it is expected that as a result, endovascular neurosurgeons will be intimately involved in the treatment of stroke patients in the futures. And finally, ultrasound enhanced thrombolysis, developed by Alexandrov et al., using high-frequency ultrasonography to accelerate enzymatic fibrinolysis by increasing penetration of drug molecules into the clot, may offer an enhanced noninvasive option of effective thrombolysis.

Promising preclinical studies and early phase clinical studies, such as the work with minocycline, indicate a potential protective effect associated with microglial inhibition. Similarly, astrocytic modulation via administration of arundic acid may result in improved outcome. Other areas of investigation with regards to inflammation, such as understanding how to manipulate macrophage polarization for therapeutic benefit may lead to breakthroughs in drug development. Lastly, biomarker discovery, while often thought of in a diagnostic or prognostic sense, may in fact serve a much broader role in understanding disease pathophysiology and therapeutic selection. By improving preclinical models to more accurately emulate the clinical scenario and targeting disease pathophysiology beyond the nearly immediate events, the likelihood of successful translation from laboratory to clinic may be improved.

Neurointerventional surgery for the treatment of ischemic stroke is a rapidly expanding field with constantly improving tools and ever evolving indications. Recanalization rates

appear to be on the rise in more recent studies compared to early studies. As data continues to accumulate regarding new treatment modalities and technologies, neurosurgeons, radiologists and neurologists should come together to design new treatment paradigms. Much work remains to streamline the care of stroke patients so that appropriate patients can be treated in a timely manner. The advances described above reflect progress across the spectrum of therapeutic options in stroke, from primary prevention to neurorepair. Every step takes us towards the ultimate aim of better outcomes and quality of life for patients with stroke.

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Chapter 15

STEM CELLS AND TREATMENT OF STROKES

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ABSTRACT

Stroke is a leading cause of death and disability worldwide. Stem cell therapy is an emerging therapeutic modality with evidence of significant benefits in preclinical stroke models. A number of phase I and II clinical trials have now been completed, with several more currently under way. Translation to the bedside, however, remains a long way off, and there are many questions that remain unanswered. This chapter will summarize the current evidence and ongoing clinical trials worldwide, and explore the challenges to making this a realistic treatment option for the future. Despite advances in acute care and secondary preventive strategies, stroke remains a major burden on health-care resources worldwide. Thrombolysis with tissue plasminogen activator is now a well-established treatment for acute ischemic stroke and is associated with significant improvements in outcomes. However, its use is limited to a narrow time window of only 4.5 hours from symptom onset, and in the UK only 5% of stroke patients currently receive this treatment. Stem cell therapy is an emerging therapeutic modality in the treatment of stroke. Its basis stems from the observation that certain parts of the adult brain are capable of regeneration (a relatively recent discovery). Neurogenesis in the adult brain has been demonstrated in the dentate nucleus of the hippocampus and the subventricular zone. In a study of patients with ischemic stroke, neurogenesis was demonstrated in the ischemic penumbra, where cells were found to preferentially localize to the vicinity of blood vessels. These findings are suggestive of poststroke compensatory neurogenesis, which may contribute to recovery after the insult. While the regenerative capacity of certain parts of the brain has been demonstrated, it is clear that this endogenous repair process is unable to overcome

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the devastating damage to brain tissue that occurs after acute, severe stroke. Cell-based therapies have the potential to open up new avenues of treatment in this arena. Targets for stem cell therapy include neuroprotective approaches aimed at protecting at-risk tissue during the acute phase of stroke, as well as neuroreparative approaches which may involve direct replacement of damaged brain tissue, or alternatively promotion of the brain's endogenous repair processes.

INTRODUCTION

Stem cells (SCs) are characterized by self-renewability, i.e., the ability to divide themselves rapidly and continuously and to create new SCs and progenitors more differentiated than the mother cells. SCs show high plasticity, i.e., the ability to transform into cells from various different tissues. The plasticity can be explained by trans-differentiation (direct or indirect) and fusion. Trans-differentiation is the acquisition of the identity of a different phenotype through the expression of the gene pattern of other tissue. By fusion with a cell of another tissue, a SC can express a gene from that cell and acquire a phenotypic element of another tissue [Zhonw W 2008, Fortier LA 2005]. In the human body, adult stem cells (ASCs) maintain the tissue homeostasis. ASCs usually differentiate in a restricted range (multi-potent) of progenitors and terminal cells to replace old and damaged cells. SCs derived from early human embryos (Embryonic stem cells (ESCs)), on the other hand, are pluri-potent and can generate all committed cell types [Perra MF 2000, Pessina A 2006]. Fetal stem cells (FSCs) derive from the placenta, membranes, amniotic fluid or fetal tissues.

Today living in the 21st century, we still do not have proper treatments for many neurological diseases like Parkinson's disease, Alzheimer's disease, etc. Some light of hope for the treatment of these incurable diseases is - the stem cells. Stem cells are a special kind of cell that has the ability to divide indefinitely and have the potential to give rise to specialized cells (that is, any cell of the body). All stem cells regardless of their source have three general properties

- They are capable of dividing and renewing themselves for long periods
- They are unspecialized
- They can give rise to specialized cell types

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

Broadly speaking, clinical approaches to stem cell therapy can be divided into “endogenous” and “exogenous” approaches:

- 1) The endogenous approach aims to stimulate mobilization of stem cells already present within the individual. Examples of this approach include the use of granulocyte-colony stimulating factor (G-CSF) used to mobilize hematopoietic stem

cells into the peripheral blood. G-CSF use has been shown to have both neuroprotective as well as neuroregenerative properties, [Shabitz WR 2003, Shyu WC 2004] and additionally appears to have direct effects beyond simply mobilization of stem cells.

- 2) The exogenous approach involves transplantation of the patient with stem cells delivered locally (e.g, direct intracerebral implantation) or systemically (eg, intravenous or intra-arterial) and may involve in vitro culture of cells for the expansion of cell numbers prior to administration. There is a large body of preclinical data and now mounting data from clinical trials that have utilized exogenous approaches to stem cell therapy for stroke. In this chapter, we will discuss these, and the challenges posed in translating this therapy to the bedside, in more detail. Of note, the vast majority of work has focused on ischemic rather than hemorrhagic stroke, and therefore we refer to data from ischemic stroke studies, unless stated otherwise.

STEM CELL THERAPY IN STROKE PATIENTS: THE CHALLENGES

Significant advances have been made in the field of stem cell therapy for stroke, and the potential benefits of such treatments are vast. However, several important questions remain unanswered, and translation to the bedside remains distant. The important areas of uncertainty are discussed below.

Table 1. Showing the Potency of Stem Cells

Stem Cell Type	Description	Examples
Totipotent	Each cell can develop into a new individual	Cells from early (Morula) (1-3 days) embryos
Pluripotent	Cells can vary form any (over 200) cell types	Some cells of blastocyst (5 to 14 days)
Miltipotent	Cells differentiated, but can form a number of other tissues	Fetal tissue, cord blood, and adult stem sells
Oligopotent	Cells differentiated, but can give rise to few different cells	Lymphoid and myeloid stem cells
Unipotent	Cells can give rise to their own type only	Mucles stem cells

WHICH STEM CELLS SHOULD BE USED?

Stem cells can be defined as clonogenic cells that have the capacity to self-renew and differentiate into multiple cell lineages [Weissman II 1998]. The two major types of stem cells, as classified by source, are embryonic stem cells (ESCs) and adult stem cells (Figure 2). Human ESCs are pluripotent and are isolated from 5-day-old human blastocysts. However, several factors limit the widespread use of ESCs. These include ethical concerns regarding the

use of unwanted embryos and concerns regarding tumorigenicity, specifically the risk of teratoma formation in vivo [Thomson JA 1998]. In contrast to ESCs, adult stem cells are multipotent stem cells that can be obtained from adults as well as children, including from umbilical cord blood. They are present within various organs, including the brain and bone marrow, and have the advantage of being suitable for autologous as well as allogeneic use. Of note, one disadvantage of these cells is that they have less differentiation potential than ESCs.

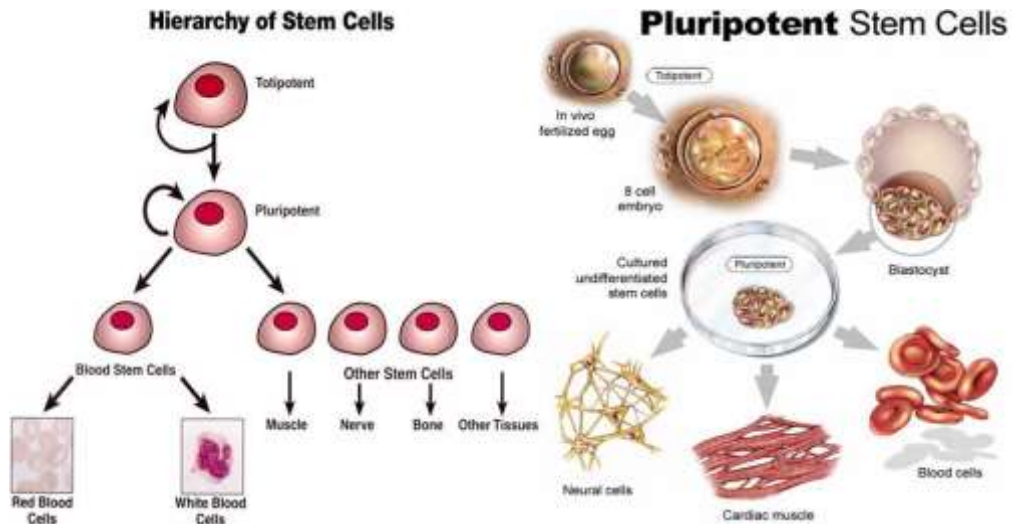
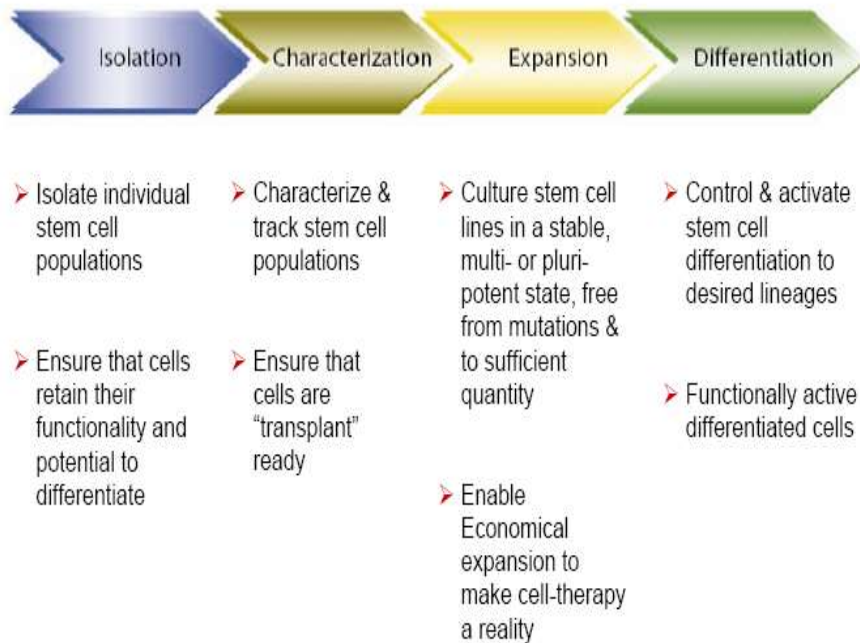
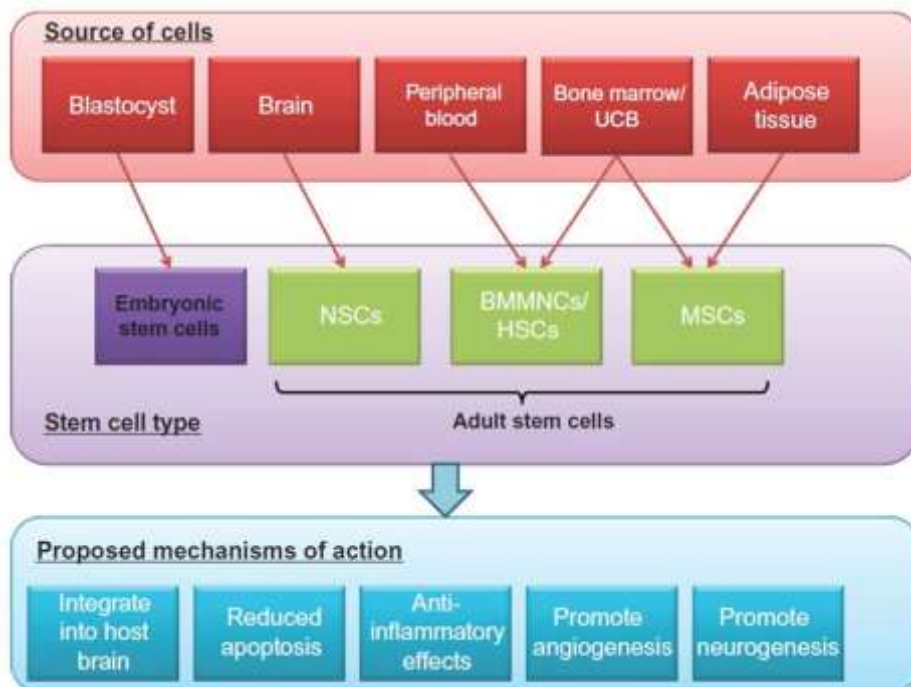


Figure 1. Stem cells can give rise to any cell type in the body. All stem cells, regardless of their source have three general properties: they are capable of dividing and renewing themselves for long periods; they are unspecialized; and they can give rise to specialized cell types.

A number of different types of stem cells, obtained from different sources, have been shown to improve clinical and radiological outcomes in preclinical models of stroke. The choice of cell type for clinical trial use should consider not only efficacy, but also ease of obtaining the cells, issues regarding cell culture for expansion, the need for immunosuppression and questions regarding dosage. A number of autologous and allogeneic cells have been tested in the preclinical arena. Issues specific to autologous cells include questions regarding extraction of consistent, adequate numbers of cells, as well as potency of the cells obtained (e.g., potency may fall with the age of the donor). Allogeneic cells must have the capacity for expansion to provide adequate numbers for widespread use, while ethical issues regarding the source will also need to be considered when using ESCs or fetal tissue, as previously mentioned. Cell-culturing techniques used for autologous or allogeneic cells will need to be consistent and adhere to strict safety criteria (good manufacturing practice). Long-term biological safety of any cell-based therapy is a further area of great importance. There is therefore a host of factors to consider when deciding which cell type is best to use. The different types of cells tested in preclinical studies are discussed in more detail below.



(A)



(B)

Abbreviations: UCB, umbilical cord blood; NSC, neuronal stem cell; BMMNC, bone marrow mononuclear cell; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell.

Figure 2. A) Stages in using Stem cells for Therapeutic Use. B) Stem cell types used in stroke trials, and the proposed mechanisms of action.

NEURAL STEM/PROGENITOR CELLS

Neural stem cells (NSCs) have the capacity to differentiate into neurons, astrocytes, and oligodendrocytes. There are a wide variety of sources of exogenous neural stem cells, with much work being done on transplantation of embryonic and fetal-derived NSCs in experimental stroke models [Chu K 2003, and 2004 Jeong SW 2003, Kelly S 2004, Hicks AU 2009]. However, the use of such embryonic or fetal tissue is limited by ethical/legal implications, in addition to important biological questions regarding the long-term risk of teratoma formation by ESCs. The emergence of induced pluripotency, whereby skin fibroblasts from patients can be transformed into ESC-like cells, may overcome the ethical issues of using fetal or embryonic tissue in future studies, while also providing a source of autologous cells, thereby removing any questions regarding immune rejection [Takashi K 2006 and 2007, Yu J 2007]. Neural stem cells derived from the adult central nervous system are a possible alternative source of cells for transplantation. Studies have documented the isolation of NSCs from the adult rodent brain [Wachs FP 2003] as well as (more recently) the adult human brain [Nunes MC 2003]. One study demonstrated that adult NSCs derived from the subventricular zone of young adult rats could survive and migrate towards the lesion in rats with an ischemic stroke following intracisternal administration [Zheng ZG 2003]. Electron microscopy examination also suggested that the transplanted cells showed signs of neuronal differentiation.

Although possible in principle, brain biopsies for isolation of adult human NSCs and autologous transplantation poststroke is technically difficult, and no clinical trials utilizing adult NSCs have been undertaken. A number of human immortalized cell lines have been shown to improve functional outcomes after experimental stroke in rodent models. These cell lines have the advantage of providing an abundant source of cells for transplantation, available ready-prepared, at the time of a stroke. Biological safety of such cell lines, however, remains a concern in the long term. The immortalized cell line NTera-2 (NT2) was derived from a human teratocarcinoma [Andrews PW 1984]. After several weeks' exposure to retinoic acid and mitotic inhibitors, these cells differentiate into postmitotic neuron-like cells, named NT2N cells (also known as hNT neurones).²³ Terminal differentiation of these cells only occurs following transplantation into an adult brain.²⁴ The transplantation of NT2N cells into the ipsilateral striatum of ischemic rat brains improved functional recovery, with some parameters of behavioral improvement persisting for up to 6 months post-transplantation [Klepner SR 1995, Borlongan CV 1995]. No evidence of tumorigenicity was found following post-mortem examination of the rats. The CTX0E03 cell line developed by ReNeuron (Guildford, UK) is a fetally derived multipotent neural stem cell line that is conditionally immortalized with c-myc-ER, such that proliferation only occurs in the presence of the drug 4-hydroxy-tamoxifen [Stromer P 2008]. It has been shown to effect a dose-dependent improvement in sensorimotor function in rodent models of middle cerebral artery ischemic stroke when delivered intracerebrally [Taguchi A 2004].

HEMATOPOIETIC STEM/PROGENITOR CELLS

Hematopoietic stem cells (HSCs) can be obtained from bone marrow, peripheral blood, or umbilical cord blood. The advantage of such cells is that they are suitable for both autologous as well as allogeneic use, and furthermore are not associated with the ethical issues surrounding ESC or fetally derived stem cells. It is contentious whether these cells can differentiate into neuronal cells. However, preclinical studies of CD34+ cells have shown significant benefits in rodent models of stroke, with evidence of functional improvement as well as reduced infarct volume. Potential disadvantages of these cells include issues with consistency of numbers and potency of cells obtained from the bone marrow, as well as the need for ex vivo expansion of cells, when using umbilical cord blood as the source.

In one study, CD34+ cell transplantation delivered intravenously resulted in increased perilesional angiogenesis and subsequent neurogenesis in mice at 48 hours poststroke [Taguchi A 2004]. A further study investigating direct intracerebral implantation of CD34+ cells 1 week after induced stroke was also able to show evidence of neurogenesis and angiogenesis, with differentiation of transplanted cells into cells expressing markers for neurons, glial cells, and vascular endothelial cells [Shyu WC 2006].

MESENCHYMAL STEM CELLS

Mesenchymal stem cells (MSCs), also known as bone marrow stromal cells, can be obtained from bone marrow as well as such other sources as adipose tissue. As with HSCs, they are suitable for autologous as well as allogeneic use. One disadvantage of this cell type is the requirement for several weeks of cell culture to generate sufficient numbers, thereby making the use of autologous MSCs in the acute phase of stroke an unviable option.

These cells have shown significant functional benefits in rodent models of stroke in the acute as well as chronic setting (up to 1 months poststroke) and when delivered by a variety of routes (intravenous, intra-arterial and intracerebral) [Chen J 2001, Shen LH 2007, Li Y 2001]. The mechanism of improvement remains unclear, though promotion of endogenous neurogenesis, reduction of apoptosis, and induction of angiogenesis have been demonstrated in different studies [Chen J 2003].

BONE MARROW MONONUCLEAR CELLS (BMMNCs)

Mononuclear cells obtained from bone marrow contain populations of both mesenchymal and hematopoietic stem cells. They have been shown to reduce neurological deficits as well as infarct size in animal models of stroke [Brenneman 2010, Giral-di-Guimareas A 2009]. These cells are easy to obtain from bone marrow, and are suitable for autologous and allogeneic use. Transplantation with HSCs (CD34+ cells) or MSCs provides purer, more concentrated cell populations, though BMMNCs have the advantage of being easier to prepare and separate from bone marrow, making them theoretically more practical, particularly in the acute setting. As with HSCs, there are issues with obtaining consistent numbers of cells from bone marrow aspiration and questions regarding the potency of cells,

particularly in older patients [Li TS 2009]. Potential mechanisms of action include paracrine effects secreting trophic factors (such as vascular endothelial growth factor [VEGF] and stromal cell-derived factor-1), as well as possible immunomodulatory benefits [Sharma S 2010].

MECHANISM OF ACTION OF STEM CELL THERAPY

The possible mechanisms of stem cell therapy include the formation of neuronal cavity, reduced apoptosis, promotion of angiogenesis and reduced inflammation. Elucidation of potential mechanisms of action is vital to the further development of stem cell therapy techniques and ultimately translation to the bedside. There are a number of proposed mechanisms that have been investigated in preclinical stroke models (Figure 1). There is very little evidence that adult stem cells integrate into the ischemic brain and form functional neurons with adequate synaptic connections. Most studies have shown very limited numbers of engrafted cells, with functional benefits in excess of what would be expected based on the degree of engraftment. Following an ischemic stroke, neurons and glia die by a mixture of necrosis and apoptosis. Cell transplantation may elicit a neuroprotective response by rescuing the apoptotic cells, particularly in penumbral tissue. This is likely to be mediated by the secretion and upregulation of certain trophins, such as basic fibroblast growth factor, brain-derived neurotrophic factor, VEGF, glial cell-line-derived factor, and nerve growth factor. Several studies have suggested that a reduction in apoptosis in the ischemic boundary area occurs following cellular therapy that is associated with improved neurological recovery in experimental models [Kurozumi K 2005, Llado J 2004]. Immunomodulatory effects of transplanted cells may have a role in neuroprotection. Preclinical studies have shown evidence of upregulation of anti-inflammatory cytokines and attenuation of expression of proinflammatory cytokines in both ischemic and hemorrhagic stroke [Lee ST 2008, Jiang Q 2005]. If an anti-inflammatory effect is to play an important role, delivery of the cells will need to be in the early stages of the stroke. Following a stroke, regenerative strategies should be aimed at not only restoration of neural elements, but also supporting structures such as blood vessels. Angiogenesis have been demonstrated after cell transplantation with various stem cells, including NSCs, CD34⁺ cells and MSCs. Direct incorporation of the transplanted cells into new blood vessels has also been demonstrated. However, more likely mechanisms of action include the release of VEGF and also the increase in endogenous levels of other factors (such as brain-derived neurotrophic factor and fibroblast growth factor) that play a role in neovascularization [Jiang Q 2005, Cheng X 2011].

PROMOTION OF NEUROGENESIS AND OTHER ENDOGENOUS REPAIR PROCESSES BY STEM CELL THERAPY

Endogenous neurogenesis is increased per se after a stroke, likely reflecting an intrinsic host-repair mechanism [Arvidsson A 2002]. Administration of cord blood cells and mesenchymal cells have been reported to enhance endogenous neurogenesis, [Nakatomi H 2002] although whether this is likely to be functionally significant is still unclear. It is

possible that transplantation of various stem cells may amplify endogenous plasticity responses, again mediated by the secretion of trophic factors. One study suggested that intracarotid administration of MSCs facilitated host neuronal and synaptic plasticity, with increased synaptophysin expression in the ischemic penumbra [Shen LH 2006]. Furthermore, axonal sprouting from the nonischemic to the contralateral hemisphere was observed following the intravenous administration of human cord blood cells [Xia J 2005].

Other stem/progenitor cells are also increased following cerebral ischemia. Under physiological conditions, a relatively small pool of CD34+ stem/progenitor cells circulates in the peripheral blood. An Ischemic stroke is known to increase levels of CD34+ cells (mobilized from the bone marrow) in the peripheral blood [Paczkowska E 2005]. In addition, there is evidence that increased CD34+ mobilisation into the peripheral blood in patients with acute ischemic stroke correlates with neurological recovery, with high counts associated with better recovery [Dunac A 2007]. Treatment with G-CSF, for example, which mobilizes CD34+ cells into the peripheral circulation, may potentially enhance this endogenous repair mechanism.

BENEFITS OF STEM CELL THERAPY TO PATIENTS

A crucial question for upcoming clinical trials is which patients will benefit from stem cell therapy. The pathological type and anatomical location of the stroke are important issues, which are discussed in more detail below. In addition, demographic differences, in particular differences in age and gender, are also likely to affect how patients respond to treatment.⁵⁴ The vast majority of preclinical work has been conducted on young, previously healthy, male animals. Elderly patients, who represent the largest subset of stroke victims, may not derive the same benefit as that seen in such animal studies. As stated previously, the majority of preclinical work has focused on ischemic rather than hemorrhagic strokes. In view of this, the ongoing and completed clinical trials have almost exclusively concentrated on ischemic stroke patients. Of the published clinical trials to date, only one has included any patients with hemorrhagic strokes [Kondziolka D 2005].

Within the ischemic stroke subgroup, the majority of preclinical evidence for benefit has been obtained from animal models with predominantly striatal infarcts. A smaller number of trials have investigated cortical models of stroke, with mixed results [Hicks AU 2008]. Cortical strokes are associated with larger, more damaging infarcts, and it may be that these are too large to affect any repair. A direct comparison between subcortical and cortical infarcts in addition to various doses of cells would be useful in order to optimize patient selection. For example, one preclinical study that compared anatomical location found the benefit of stem cell therapy to be more pronounced in striatal infarcts, rather than larger cortical lesions [Smith EJ 2012]. Further issues to consider when designing clinical trials are stability of patients and associated comorbidities. Patients with larger strokes are commonly medically unwell due to multiple reasons (eg, aspiration pneumonia, cardiac arrhythmias), which could preclude them from entry into acute trials despite being eligible. Furthermore, multiple comorbidities are also likely to affect the response of the patient to stem cell therapy.

OPTIMAL TIMING FOR TREATMENT

Preclinical studies have shown benefits of stem cell therapy in both acute and chronic models of stroke. Decisions regarding the ideal timing need to take into account the likely mechanism of action. A neuroprotective mechanism would require acute delivery, although graft survival may be affected by the damaging effects of ischemia and reperfusion, mediated via excitotoxic neurotransmitters, free radicals, and other inflammatory mediators [Mitsios N 2006]. If the aim is to enhance endogenous repair mechanisms and inhibit neuronal apoptosis, then acute or subacute delivery would be desirable, as these processes occur predominantly in the first few weeks postischemia [Hayashi T 2003]. A neuroreparative approach aiming for direct replacement of damaged tissue with new neuronal circuitry (using intracerebral transplantation), would benefit from a later timescale, once neuroinflammation has subsided. Such a strategy would be looking at treatment delivered weeks to months after the stroke, in keeping with ongoing host plasticity during that time.

Future studies will need to address questions regarding timing, with direct comparisons between acute and chronic delivery. One animal study demonstrated significant neurological improvements with intravenous injection of BMMNCs at 24 and 72 hours, poststroke but showed no improvement in animals treated at 7 days poststroke [Yang B 2011]. Other studies comparing acute to chronic delivery of cells have shown conflicting results, though there were important differences between these trials in terms of the cells used, animal model, and exact timing of transplantation. [Grabowski M 1994, Darsalia V 2011] Such comparative data is sparse in the preclinical arena and sorely lacking in the clinical arena, and at present the optimal timing of treatment is yet to be determined.

DELIVERY ROUTE OF STEM CELLS

Studies in the preclinical and clinical settings have used a variety of different routes of stem cell delivery, illustrating the point that the best delivery method remains unclear. Benefits have been seen using direct intracerebral implantation as well as systemic delivery using the intravenous and intra-arterial routes. Intracerebral implantation allows direct delivery of cells to the affected site, but is invasive and may present issues of disrupting the underlying brain tissue. Use of a general anesthetic for intracerebral delivery, particularly in the acute stroke patient, is problematic, though alternatively a local anesthetic can be used. The intravenous route is the safest, easiest route of delivery, though there are issues regarding accumulation of cells in nontarget organs, with only a small proportion reaching the brain [Fisher UM 2009]. The size of the cells used is important in this regard. The intra-arterial route allows more direct delivery of cells, allowing better first-pass delivery while being less invasive than direct surgical implantation.

Decisions regarding the route of delivery will need to take into account issues regarding the practicality and safety, as well as cell type/dosage and proposed mechanism of action. The best route of delivery is yet to be determined, and there is a paucity of preclinical data comparing the different available delivery methods. One study of ischemic stroke in rats compared intravenous to intra-arterial delivery of neural progenitor cells. Their data showed a superior cell delivery to the ischemic hemisphere, with the intra-arterial route [Lappalainen

RS 2008]. However, as mentioned previously, all three modes of delivery have clearly shown benefits in different preclinical studies, which raise the question: is it necessary for cells to enter the brain to have their beneficial effect? More well-designed trials are needed to directly compare different routes of delivery using standardized criteria, in order to help answer these vital questions.

How Can Transplanted Cells Be Tracked?

Tracking of cells is paramount to improving our understanding of cell migration and mechanisms of action. Currently there is no ideal method of doing this, with the techniques available being suboptimal for a number of reasons.

One commonly employed tracking method in preclinical studies is cell labeling with superparamagnetic iron oxide particles, which allows the anatomical location of transplanted cells to be determined using magnetic resonance imaging (MRI) [Bulte JW 2002]. Other examples of imaging modalities used to track labeled cells include positron emission tomography (PET), bioluminescence imaging (BLI), and single-photon-emission computed tomography (SPECT) [Love Z 2007]. One drawback of many cell-labeling techniques is the difficulty tracing the labeled cells when they divide, either due to failure of uptake of the label or the presence of a weaker signal in the new cells. In addition, if the cell dies and is phagocytosed by macrophages, any signal received will be misleading [MrColgan P 2011]. BLI overcomes this issue, as it picks up luminescence signals emitted from living cells. However, one of the concerns with BLI is poor spatial resolution, an important drawback of this modality [Bliss T 2010].

To date, only one clinical trial of stem cell therapy in stroke patients has investigated the role of tracking the transplanted cells. A study of BMMNCs labelled with technetium-99m (^{99m}Tc) looked at cell homing and uptake using whole-body scintigraphy and SPECT scans [Barbossa LM 2010]. They confirmed the presence of cells in the brain at 2 hours, as well as distributed to the liver, lungs, spleen, kidneys, and bladder. Furthermore, preferential accumulation of radioactivity was seen in the ipsilateral affected hemisphere (compared to the contralateral hemisphere) in all patients at 2 hours postinfusion. In addition to tracking cells, imaging modalities are also required to evaluate the functional outcome of transplanted cells. Diffusion-weighted MR studies can be used to assess for neovascularization, PET scanning to assess metabolic changes, diffusion-tensor imaging to assess fiber-tract integrity, and functional MRI to assess neuroplasticity. It is likely, therefore, that a combination of imaging modalities will eventually be required to give a better overall assessment of the fate of transplanted cells, in order to further the understanding of their mechanisms of action.

Stem Cell Therapy in Stroke Patients: Clinical Trials

Clinical trials of stem cell therapy in stroke patients are still in their infancy. There have been a number of recent phase I/II trials, primarily investigating the role in ischemic stroke patients. However, these trials have not clearly defined the best cell type, route of delivery, or timing of therapy, and more coordinated research is needed to better answer these uncertainties.

The majority of preclinical work has focused on treatment in the acute or subacute phases of stroke. In contrast, all the early clinical trials have concentrated on chronic stroke patients. There is certainly preclinical evidence pointing to benefit in the chronic subgroup. In addition, patients are more stable and chronic delivery occurs well after the acute inflammatory response has subsided. There are now an emerging number of acute human trials that have also been completed. Reasons for acute delivery of cells have been discussed previously. It is important to note that the pathophysiological processes and proposed mechanisms of action in the acute phase are quite different to the chronic phase of stroke, and it is not possible to extrapolate data gleaned from one setting to the other. Future studies will need to address questions regarding the ideal timing of transplantation.

The currently available clinical trial data utilizing human stem cells will be discussed further, as well as a discussion on ongoing trials, of which there are now several scattered across different centers worldwide.

CHRONIC STROKE TRIALS

NT2N Cells

NT2N were the first human cells to be tested in stroke clinical trials. A phase I trial examined the safety of direct stereotactic transplantation of cells into patients with basal ganglia stroke that had occurred 6 months to 6 years previously [Kondziolka D 200]. Twelve patients with stable motor deficits were treated, along with an immunosuppressive regimen of cyclosporine-A due to the allogeneic nature of the treatment. Two doses of cells were used: 2×10^6 and 6×10^6 cells. There was a trend towards improved functional outcomes in the four patients who received the higher dose of cells. Autopsy on one patient, who died of myocardial infarction 27 months post-transplantation, showed apparent survival of NT2N cells in the brain. Furthermore, PET scans at 6 months post-transplantation showed increased metabolic activity at the site of infarct in six patients [Nelson PT 2002]. This initial safety trial led to a phase II study assessing the effect of NT2N cell transplantation in 18 patients with a basal ganglia stroke between 1 and 6 years previously. Stereotactic cellular transplantation plus two months of rehabilitation ($n = 14$) was compared to control patients who received rehabilitation alone ($n = 4$). Six of the 14 patients who received a transplant appeared to show improvement on a standardized stroke scale, but this was not statistically significant against control patients. Of note, this trial included ischemic ($n = 9$) and hemorrhagic strokes ($n = 9$).

Mesenchymal Stem Cells

The extensive preclinical work on MSCs described previously led to a phase I/II clinical trial using autologous culture-expanded MSCs in patients with ischemic middle cerebral artery (MCA)- territory stroke.⁷⁵ Thirty patients with established stroke (1 month postinfarct) were randomized to receive MSCs by the intravenous route ($n = 5$) or no MSCs ($n = 15$). Cells were extracted by bone marrow aspiration and culture-expanded prior to infusion. There

was no cell-related toxicity related to the MSC administration, and the authors suggested that functional improvement may have been better in the transplanted group compared with the control group, although this was not statistically significant. There were no significant differences between the two groups in relation to changes in infarct volume at 1-year follow-up. Following on from this study, the same group conducted a trial looking at the long-term (5-year) effects of MSC transplantation in patients with severe MCA-territory stroke [Lee JS 2010]. Sixteen patients (versus 36 controls) were treated with intravenous delivered, autologous, culture-expanded MSCs. There was no difference in adverse events between the two groups. Functional outcome appeared to be better in the treated group, with a significantly increased proportion of patients with a modified Rankin score of 0–3, compared to the controls.

A further trial of autologous MSCs administered in ischemic stroke patients, within 6 months of their event, was recently reported [Honmou O 2011]. Cells were delivered intravenously 36–133 days poststroke in an open-label study of 12 patients. MSCs were serum-expanded prior to infusion. The treatment was shown to be safe and feasible, with no significant adverse events. Furthermore, mean infarct volume as assessed by MRI was reduced by .20% at 1 week postinfusion. Of note, the culturing conditions and media were different between the two MSC trials described above.

Bone Marrow Mononuclear Cells

Two trials utilizing BMMNCs have been reported in chronic stroke patients. One open-label study treated six male patients with the MCA-territory ischemic stroke within 90 days of the event.⁷⁸ Autologous BMMNCs were isolated after bone marrow aspiration. The cells were then delivered intra-arterially, with a proportion of these labeled with ^{99m}Tc. There were no treatment-related adverse events up to 6 months follow-up. Evidence was seen of cell migration in the brain at 2 hours postinfusion in all six patients, though at 24 hours this number had dropped to two patients [Barbosa LM 2010]. All patients showed improvements in their National Institutes of Health Stroke Scale scores by 6 months. In a separate study, autologous bone marrow stem cells obtained by bone marrow aspiration were transplanted stereotactically in five patients with established stroke [Saurez C 2009]. The procedure was well tolerated, with no adverse events up to 1 year follow-up.

Ongoing Chronic Trials

There are at least ten other ongoing or completed trials in chronic (4 weeks post event) stroke patients that are yet to report. Details of these unreported trials are listed in Table 5.2. A number of different cell types at different time points and via different methods of delivery are being tested. These include allogeneic cells, such as the CTX0E03 neural stem cell line, which has been discussed previously.

Such commercially developed cell lines, if shown to be beneficial, are an exciting prospect owing to the ability to develop them for “off the shelf” use, available ready-prepared at the time of a stroke. The results of these various trials are awaited with interest, though it is clear to see that meaningful comparisons between the different trials will be difficult to make,

and how far these trials will go towards answering the outstanding questions in this field is difficult to say.

ACUTE STROKE TRIALS

Bone Marrow Mononuclear Cells

There are currently three published trials of stem cell therapy in acute stroke patients. All three trials utilized BMMNCs and were able to demonstrate safety and feasibility in the treated subjects. One study treated ischemic stroke (MCA territory) patients at 24–72 hours post event with intravenously delivered cells [Friedrich M 2012]. Ten patients were treated in an open-label study, with no evidence of study-related serious adverse events up to 6 months follow-up. All patients had shifted down by at least 1 point on the modified Rankin Scale (mRS) at 6 months compared to at day 7. The second study (also open-label) treated 20 patients with MCA-territory ischemic stroke at 3–7 days post event, with the cells delivered intra-arterially [Friedrich M 2010]. There were no procedure-related adverse events reported up to 6 months follow-up. A good outcome (defined as an mRS of #2) was seen in eight of the 20 patients at 90 days. The third, most recently published trial treated ten patients with BMMNCs delivered intra-arterially at 5–9 days after stroke [Monichie F 2012]. This was a single-blind, controlled study (ten consecutive controls; nonrandomized). Two of the treated patients had isolated partial seizures at 3 months. There were no significant differences in neurological outcome between the two groups at final 180-day follow-up. None of these trials were powered to demonstrate efficacy.

Ongoing Acute/Subacute Trials

A further six acute/subacute trials (4 weeks poststroke) are ongoing or yet to report (Table 5.3). As with the chronic stroke trials, these are utilizing a variety of different cell types, including BMMNCs, CD34⁺ cells, and MSCs, as well as commercially developed stem cell lines. We have investigated the use of intra-arterially delivered CD34⁺ cells in patients with severe MCA-territory ischemic stroke, recruited within 7 days of the event (ClinicalTrials.gov identifier NCT00535197). To date, no trial has reported on the effects of immunoselected CD34⁺ cells for acute or chronic ischemic stroke. Such cells have been shown to improve functional outcome and infarct size in preclinical models. Furthermore, a recently published trial of BMMNC treatment of acute ischemic stroke showed a trend towards a positive correlation between number of CD34⁺ cells injected and functional outcome at 1 month [Monichie F 2012]. We have demonstrated safety and feasibility in our patient cohort, as well as improvements in clinical scores and functional status at 6-month follow-up (unpublished data). Of the other ongoing studies, one is investigating the use of MSCs in hemorrhagic as well as ischemic strokes. This will be of interest, as the use of stem cells for hemorrhagic stroke has not been well investigated in the clinical arena. It must be borne in mind however, that the underlying pathophysiological processes at play and therefore the mechanisms of recovery are quite different between these subtypes of stroke. The completed results of these various trials are eagerly awaited.

Table 2. Summary of ongoing or completed (but unreported) chronic stroke trials

Cell type	Study design	Expected no of patients	Timing of delivery post stroke	Route of delivery	Clinical trial identifier	Trial status
Chronic trials (4 weeks post event)						
CTX0E03 neural stem cells	PI, NR-OL	12	6 months–5 years	IC	NCT01151124	Recruiting
MSCs	PII, R-OL	30	,6 weeks	IV	NCT00875654	Recruiting
CD34+	PII, R-OL	30	6–60 months	IC	NCT00950521	Complete
CD34+	PI, NR-OL	6	6–60 months	IC	NCT01438593	Not yet recruiting
CD34+	PI, R-OL	40	,1 year	IA	NCT01518231	Recruiting
MSCs, EPCs	PI/II, R-DB	90	5 weeks	IV	NCT01468064	Recruiting
MSCs	PII, R-OL	50	1 week–2 months	IV	NCT01461720	Recruiting
MSCs	PI/II, NR-OL	35	.6 months	IV	NCT01297413	Recruiting
SB623	PI/II, NR-OL	18	6–36 months	Unclear	NCT01287936	Recruiting
OECs	PI, R-SB	6	6 months–5 years	IC	NCT01327768	Recruiting

Note: CTX0E03 and SB623 are commercially developed stem cell lines.

Abbreviations: BMMNC, bone marrow mononuclear cells; MSCs, mesenchymal stem cells; OECs, olfactory ensheathing cells; EPCs, endothelial progenitor cells; NR, nonrandomized; OL, open-label; PI and II, phase I and II trials; R, randomized; SB, single-blind; DB, double-blind; IV, intravenous; IA, intra-arterial; IC, intracerebral.

THE FUTURE

Routine use of stem cells at the bedside for stroke patients is an exciting prospect, though realistically remains a long way off. The future may well see the availability of “off-the shelf” stem cell products available for immediate use. Perhaps patients will receive individually tailored stem cell products, engineered to secrete specific trophic factors, to suit their specific subtype and duration of stroke. There is clearly a long way to go in both preclinical and clinical research, and long-term biosafety measures will be an essential consideration in this regard.

A number of clinical trials have now begun to demonstrate the safety and feasibility of different approaches to stem cell therapy. However, there are a large number of unanswered questions that remain. The great variation in the stem cell trials completed to date means that it is difficult to make any meaningful comparisons between them. Future clinical trials will need to start trying to address the important outstanding questions. It is imperative that trials are therefore designed with this in mind. Careful planning and collaborative work need to take place in order to maximize the likelihood of getting a useful answer from these studies. This includes such considerations as incorporation of a dose-escalation design and comparisons between different methods of delivery as well as different time points for delivery. The Stroke Therapy Academic Industry Roundtable (STAIR) II meeting elucidated the important considerations when designing future phase IIb studies. Many of these recommendations are particularly relevant to the field of stem cell research (see Figure 2; important factors highlighted). They emphasized that “lack of establishing the optimal dose, duration of therapy, and time window may have contributed to the failure of neuroprotection trials.” Therefore, refinement and identification of the target population is essential. In addition, narrowing selection criteria in phase IIb studies to target patients more likely to respond based on clinical and imaging characteristics may optimize the chances of detecting a biologically relevant drug effect (STAIR IV recommendations). Subsequent to these publications, the Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS) meeting sought to develop a framework specifically to help guide design of future preclinical and clinical studies in the field of cell-based therapies [STEPS 2009]. These recommendations from experts from the preclinical and clinical arenas should be carefully borne in mind before commencing any future trials.

In conclusion, there have been significant advances made in the field of stem cell research over the last two decades, with evidence of significant benefits in both acute and chronic animal models of stroke. Translation to clinical practice, however, remains a long way off. A number of trials are under way, though future work will need to concentrate on overcoming the still-significant challenges standing in the way of this becoming a realistic treatment option for the future.

Table 3. Summary of ongoing or completed (but unreported) acute/subacute stroke trials

Cell type	Study design	Expected no of patients	Timing of delivery poststroke	Route of delivery	Clinical trial identifier	Trial status
Acute and subacute trials (,4 weeks postevent)						
BMMNC	PII, R-OL	120	7–30 days	IV	NCT01501773	Complete
MSCs	PII, NR-OL	120	7–14 days (infarct)or 10–21 days (ICH)	IV, then intrathecal 7 days later	NCT01389453	Recruiting
CD34+	PI/II, NR-OL	10	7 days	IA	NCT00535197	Recruiting
MSCs	PI/II, R-DB	78	,10 days	IV	NCT01091701	Not yet recruiting
MultiStem	PII, R-DB	140	1–2 days	IV	NCT01436487	Recruiting
ALD-401	PI/II, R-DB	100	13–19 days	IA	NCT01273337	Recruiting

Note: MultiStem and ALD-401 are commercially developed stem cell lines.

Abbreviations: BMMNC, bone marrow mononuclear cells; MSCs, mesenchymal stem cells; OECs, olfactory ensheathing cells; EPCs, endothelial progenitor cells; NR, nonrandomized; OL, open-label; PI and II, phase I and II trials; R, randomized; SB, single-blind; DB, double-blind; IV, intravenous; IA, intra-arterial; IC, intracerebral; ICH, intracerebral hemorrhage.

**Table 4. Stroke Therapy Academic Industry Roundtable (STAIR)
II recommendations**

Considerations for designing phase IIb stroke trials
• Route of administration
• Dose range
• Duration of treatment
• Time from stroke onset to initiation of treatment (a key variable)
• Pharmacokinetic profile
• Side effects and their frequency (with attention to side-effect management)
• Interactions with other commonly used medications
• Drug distribution to the proposed site of action
• Refinement and identification of the target population (e.g, drugs without preclinical evidence of activity in white-matter ischemia should not be studied in patients with subcortical stroke or even large cortical events with attendant subcortical injury)
• Obtain evidential measurement of therapeutic activity by evaluation of clinical and/or surrogate markers (hints of potential effectiveness)

Adapted from Stroke Therapy Academic Industry Roundtable II (STAIR-II). Recommendations for clinical trial evaluation of acute stroke therapies. *Stroke*. 2001;32:1598–1606.83 **Note:** Important factors relevant to stem cell therapy underlined.

**Table 5. Stem Cell Therapies as an Emerging Paradigm in Stroke
(STEPS) recommendations**

Considerations for animal and human testing of cellular therapy
Mechanism of action
• Trophic factors
• Cell replacement
• Local environment support
• Other
Pathological substrate
• Ischemic versus hemorrhagic
• Peri-infarct versus intra-infarct
Location of pathology
• Cortical versus subcortical
• Brain stem
Timing after stroke
• Acute
• Subacute, chronic

Adapted from Stem Cell Therapies as an Emerging Paradigm in Stroke participants. Stem Cell Therapies as an Emerging Paradigm in Stroke (STEPS): bridging basic and clinical science for cellular and neurogenic factor therapy in treating stroke. *Stroke*. 2009;40:510–515.85.

SOURCES OF TRANSPLANT CELLS

Stem cells must possess two characteristics: they must be able to replicate and they must have the capacity for differentiation into multiple cell lines [Preston SL 2003]. Stem cells vary in their degree of potential for differentiation. Totipotent cells can form the embryo, as well as any cell line. Pluripotent cells cannot form an embryo, but can differentiate into cells of multiple different tissue cell lines. Multipotent cells can only produce a limited set of cell lines, appropriate for a given tissue environment. Unipotent cells can only generate one cell type. Thus, fetal tissue can possibly yield pluripotent stem cells and certainly generate organ-specific multipotent stem cells. Of course, the use of human fetal tissue is controversial for ethical reasons and limited (in the USA) by recent legislative restrictions. Other potential sources for stem cells exist. Multipotent stem cells have been identified within adult tissue, including brain, although not in sufficient numbers to be reasonably extracted for clinical use [Gu W 2000]. In some organs, notably the bone marrow, multipotent cells have shown a capacity to “change their minds” and transdifferentiate into lineages of other organs, including neural cells [Sanchez Ramos J 2000, Woodbery D 2000]. There is evidence that human umbilical cord blood cells (HUBCs) may also manifest transdifferentiation [Sanchez Ramos JR 2001]. Finally, tumor cell lines occasionally yield lines of immortal, organ-specific, multipotent stem cells. An example of a tumor cell line that may prove useful in neurotransplantation is the Ntera 2/cl.D12 (NTs) human embryonic carcinoma-derived cell line [Kleppner SR 1995]. These cells replicate and differentiate into human neuronal cells [Trojanowaski JQ 1993]. Thus, NTs have the characteristics of multipotent neural stem cells. When transplanted, these neuronal cells survive, extend processes, express neurotransmitters, form functional synapses, and integrate with the host. The final cells appear virtually indistinguishable from terminally differentiated, postmitotic neurons. The cells are capable of differentiation to express different neuronal markers characteristic of mature neurons. Their neuronal phenotype makes them a promising candidate for replacement in central nervous system disorders, as a virtually unlimited supply of pure, postmitotic, terminally differentiated human neuronal cells.

MULTIPOTENT CELL TRANSPLANTS IN ANIMAL MODELS OF STROKE

In 1998, Borlongan et al. showed that transplants of LBS-neurons could reverse the deficits caused by a stroke (Borlongan CV 1998). They used a rat model of permanent focal ischemia. At 1 month postischemia surgery, they selected all rats that showed significant behavioral deficits and randomized this group to receive transplantation either of fetal rodent striatal cells ($n = 8$) or human embryonic carcinoma-derived (hNT) neurons ($n = 14$). They had an existing set of 22 control rats that received transplants of either cerebellar cell or cell medium. They tested all animals at 1, 2, 3, and 6 months after surgery, having noted previously that ischemic animals continue to display significant deficits for >3 months postischemia. They used the passive avoidance test (the same test used to identify rats with significant deficits poststroke) and the elevated body swing test (EBST), a measure of motor asymmetry. The rats who received either striatal fetal cells or hNT neurons significantly outperformed the controls; rats receiving hNT had a significantly earlier recovery of

behavioral deficits compared to those who received the striatal fetal cells. Similarly, rats who received hNT neurons recovered motor symmetry significantly faster than those receiving striatal fetal cells, while both of these groups significantly outperformed the controls. Immunohistochemical staining done on treatment rats showed that there was survival of the grafted cells after 6 months.

CELL TRANSPLANTS IN HUMAN STROKE

The transplantation of hNT neurons into the brains of humans with stroke has now undergone Phase I and Phase II trials, with promising results. The first clinical study of transplantation of the immortalized line of hNT cells into human stroke victims was conducted at the University of Pittsburgh. The goal was to demonstrate the safety and feasibility of the neuronal cell implantation procedure. The study was an open-label trial with observer-blinded neurologic evaluation of patients with stroke who received stereotactic implants of human neuronal cells. Patients with basal ganglia infarcts and stable motor deficits were enrolled. They had to have had strokes between 6 months and 6 years prior to transplantation. The mean time since onset of the stroke was actually 27 months (range 7 to 55). Cells were delivered stereotactically via three 20- μ l injections along a single trajectory through the infarct. The first four patients received 2 million cells via single-pass injections into the area of infarction. The next eight patients were randomized to receive either 2 or 6 million cells (three separate stereotactic trajectories with three injections each around the stroke). Because of concerns for cell rejection, the patients were given intravenous methylprednisolone during the procedure, followed by cyclosporine-A administration beginning 1 week before surgery and for 8 weeks following. Patients were evaluated for safety and efficacy at ten intervals over the 52 weeks following surgery. Disability was assessed using the National Institutes of Health Stroke Scale (NIHSS), European Stroke Scale (ESS), and Barthel Index (BI). Positron emission tomography using fluorodeoxyglucose (FDG-PET) and magnetic resonance imaging (MRI) scans were obtained at baseline; MRI was repeated at weeks 4 and 24, while PET was repeated at weeks 24 and 52. The goals of demonstrating safety were met, with no adverse events related to implantation. One patient developed renal insufficiency attributed to the cyclosporine-A. One patient, whose infarct involved temporal lobe cortical tissue, had a generalized seizure 6 months after the surgery and was treated successfully with anticonvulsant medication. One patient had a subsequent stroke, remote from the site of neuronal transplantation, 6 months after the surgery. No malignancies, deaths, or cell-related adverse events occurred in the follow-up period. Although the study was not designed as an efficacy study, the functional outcomes suggested clinical benefit. The ESS, which had been stable between the preoperative evaluation and the day of surgery, improved in 6 of the 12 patients, remained unchanged in 3, and deteriorated in 3, compared with baseline scores. The mean improvement in ESS from baseline to week 24 was 2.9 points, achieving significance ($p = 0.046$). Motor improvement accounted for much of the clinical improvement in the ESS in the patients enrolled in this first study. The NIHSS seemed to improve overall in the group, without achieving significance: eight patients had improved, one was unchanged, and three deteriorated at the 24-week follow-up. The BI did not change significantly. Serial MRI scans showed no changes in the brain after the surgery

when compared with baseline. The serial PET scans, however, manifested differences between the baseline and follow-up studies done 6 months later. There was an increase in $\geq 15\%$ in the relative uptake of FDG within the transplant site, in 6 of 11 patients. This increased metabolic activity suggests the return of viable neurons to the areas of infarction. While an inflammatory response could produce such findings, there was no sign of inflammation on serial MRI. The suggestion of cell survival in the serial PET studies was corroborated by some pathological data. One of the patients, a 71-year-old man, died of a presumed myocardial infarction 27 months following the transplantation surgery. He was one of the patients who demonstrated no motor recovery. Histochemical stains of his brain demonstrated a population of neurons at the graft site. Many of these were NF protein immunoreactive, suggesting that they were nNT neurons. This was confirmed with a special single chromosome 21-specific probe (specific for hNT neurons) that detected multiple signals in nuclei within the area of the graft. Therefore, in the Phase I study, transplantation of the hNT neurons into areas of stroke was shown to be both safe and feasible. The transplant cells survived, and there was even a suggestion of clinical benefit. A Phase II dose-response trial was undertaken at two centers (University of Pittsburgh and Stanford University). Patients were randomized. The first seven of these received 5 million cells along five trajectories with five injections each (i.e., one million cells per trajectory), while another seven patients received 10 million cells, along five trajectories and with five injections each. There were four control patients. All patients received cyclosporine for 6 months as well as focused stroke rehabilitation. The primary outcome was the motor ESS at 6 months. NIHSS, ESS, and MRI were done on routine follow-up. The safety results of the Phase II trial were similar to those of Phase I, with no major medical complications of the procedure and no lasting effects due to implantation or cyclosporine administration. One patient experienced a syncopal episode, 1 month following surgery. One patient experienced a seizure 1 day after surgery. One patient, who was on aspirin and clopidogrel combination therapy, underwent surgical drainage of a chronic subdural hematoma, 1 month following transplant. Two of the four control patients experienced improvements in their motor ESS scores after 6 months of physical therapy. The range of change on this scale was -0.5 to $+3.5$ points. Of the transplant group, 8 of the 14 improved after 6 months, and the range was -5.5 to $+14.5$. Overall, the transplant group had a statistically insignificant improvement in motor ESS over the control group ($p = 0.148$). However, the relatively large degree of improvement of some of the transplant patients was notable. Unexpected improvements were also noted in some of the secondary outcome measurements. For example, there seemed to be a trend toward improvement in the treatment group with respect to hand and wrist movement. Interestingly, there were significant improvements in certain neuropsychological measurements, such as visuospatial construction and retention, and nonverbal memory, in the treatment group compared with the controls. These results were recently published [Kondziolka D 2005, Stillely CS 2004].

From these first two trials, it is inferred that stereotactic cell implantation of immortalized multipotent neural stem cells, derived from a tumor cell line, is relatively safe and devoid of delayed cell-related adverse effects; the transplanted cells survive *in vivo*; and some patients that received cell transplants showed motor and cognitive improvements. Future trials using this cell line will have to address optimal patient selection, timing of surgery, number of cells needed, optimal implant location, and the need for immunosuppression.

POSSIBLE MECHANISMS

It remains unclear exactly how hNT neuron transplants exert their effects in animal stroke models and their possible effects in humans with stroke. There is suggestive evidence that the transplanted cells do survive. It remains to be proven whether they also integrate into the existing neural network that surrounds them, although the surprising improvement in cognition that was observed in some of the transplant patients, if there must also be other mechanisms involved. The improvements noted in the bone marrow stromal cell [Bang Oy 2005] pilot trial and in the HUBC animal models [Chen A 2001] occurred too soon to be attributable to neuronal integration. Perhaps, the transplanted cells induced a host response, such as the secretion of neurotrophic factors, which independently promoted recovery. BMSCs are known to secrete interleukins (IL)-6, IL-7, IL-8, IL-12, IL-14, IL-15, along with macrophage stimulating factor, Flt-3 ligand, and stem cell factor [Eaves CJ 1991]. These cytokines have been identified as promoters of growth and survival of mouse hippocampal neuron progenitor cells [Mehler MF 1993]. BMSCs also release neurotransmitters which can supplement for lost neurons after ischemia. It is therefore possible that these or other trophic factors could perhaps stimulate synaptic sprouting in damaged brain tissue.

CONCLUSION

Stroke is a major cause of disability, and a repair strategy needs to be developed. Cell therapies have the potential for providing a means of repairing brain tissue that has been permanently injured by stroke. There are several sources of neuronal progenitor cells that are under investigation. In animal models, it has been successfully shown that fetal cell transplants are able to survive and convey functional recovery. BMSCs and HUBCs seem to be able to transdifferentiate into neuronal cells and survive in the brains of animals with stroke. On the other hand, further research is required to determine the functional significance of this mode of transplantation. Implanted tumor-derived neuronal progenitor cells (hNT neurons) survive and convey functional improvement in animals. There is promising preliminary evidence that the same holds true for humans. Once a source of neuronal progenitor cells can be found that can consistently differentiate into viable neuronal cells and then survive within stroke-injured brain tissue, further research will then be able to refine the process. Future research would then focus on determining the ideal timing of transplantation, selection of the best size and location of the cellular implant, and possibly even optimizing the cellular environment by controlling local trophic factor concentrations. The goal is to replace lost neurons in a self-sustainable tissue milieu, and reintegrate the replacement cells into existing neural networks, in order to ultimately restore functions that have been lost.

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Chapter 16

AN INTRODUCTION TO CEREBROVASCULAR DISEASES

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ABSTRACT

Cerebrovascular disease [CVD] is the most common life threatening neurological condition, a major contributor to morbidity, functional disability and mortality worldwide. According to the recent epidemiological data, it is expected that, till 2020, coronary artery disease and cerebral hemorrhage will be still the first and second causes of death of human beings, even though the order of the death causes due to human diseases would be changed significantly. CVD are a heterogeneous group of disorders such as stroke, vascular cognitive impairment, and even certain migraine subtypes with a variable natural history. Neurovascular disease has a wide range of phenotypes involving multiple cell types of the brain, the blood brain barrier [BBB], and interaction with the peripheral systemic circulation. Unlike cardiovascular disease, which largely involves in situ vascular thrombosis, neurovascular disease can induce focal injury with thrombotic [involving larger and small vessels], embolic [from cardiac source or periphery], hemorrhagic [intracerebral and subarachnoid], inflammatory, and traumatic mechanisms, to name only a few. It can result in global ischemic injury from lack of perfusion, such as from sudden cardiac arrest. Outcome depends on many factors, including the underlying pathophysiology, collateral cerebral circulation, and concurrent illness, especially heart disease. About 80% of all cerebrovascular events are ischemic in origin, and most are associated with atherosclerotic disease. Despite various advances in the understanding of the diseases, pharmacological treatment by conventional medicine has not obtained satisfactory results. However, recent studies suggest that natural products and traditional herbal medicine are a potential candidate for the preventative treatment of the disorders. Therefore, the application of bioactives/traditional herbal medicine in prevention and treatment of cerebrovascular diseases is of great significance. A history of

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cerebrovascular disease is important because specific therapy may be indicated to prevent further events, and because the history of cerebrovascular disease may be a "marker" for other underlying disease, especially coronary artery disease. This chapter will discuss in detail the different types of CVD, their risk factors and other parameters of CVDs.

INTRODUCTION TO Cerebrovascular Diseases

Worldwide stroke and other cerebrovascular diseases [CVD] are a leading cause of physical disability. They are the second commonest cause of mortality [accounting for 10.8% of all deaths; [WHO, 2011] and the primary reason for admission in a large proportion of hospital inpatients [1]. The World Health Organization defines stroke as a rapidly developing focal [or global] brain dysfunction of vascular origin lasting more than 24 h, thus encompassing ischaemic stroke, intracerebral haemorrhage, subarachnoid haemorrhage and cerebral venous sinus thrombosis [2]. CVD is highest in the elderly and the morbidity and costs associated with age-related disorders such as these are likely to continue to mount with the pattern of extended life expectancy observed in Western populations [3]. There are around 700,000 new and recurrent strokes per year in the US, with costs related to stroke estimated to be over \$60 billion in 2007 [4]. Cerebrovascular disease remains a devastating and highly morbid disease and the third leading cause of death after heart diseases and cancer in developed countries. The economic burden of stroke is tremendous, both because of the direct costs of medical care, medications, and rehabilitation, and indirect costs such as lost time at work and caregiver burden. Cerebrovascular disease is caused by one of several pathological processes involving the blood vessel of the brain. The process may be intrinsic to the vessel as in atherosclerosis, lipophyalinosis, inflammation amyloid deposition arterial dissection, developmental malformation, aneurysmal dilation, or venous thrombosis; originate remotely, as occurs when an embolus from the heart or extracranial circulation lodge in an intracranial vessel; result from decreased perfusion pressure or increased blood viscosity with inadequate cerebral blood flow; or result from rupture of a vessel in the subarachnoid space or intracerebral tissue[5]. Cerebrovascular diseases are a heterogeneous group of disorders with a variable natural history. Outcome depends on many factors, including the underlying pathophysiology, collateral cerebral circulation, and concurrent illness, especially heart disease [6].

The risk of an ischemic event increases with age and is correlated with both systolic and diastolic blood pressure, diabetes, and a history of ischemic heart disease or previous stroke. All epidemiologic studies have identified hypertension as the most important risk factor for CVD. Correlation with cigarette smoking and hyperlipoproteinemia is less conclusive. A neurologic symptom or symptom complex caused by cerebral ischemia or hemorrhage is commonly called a cerebrovascular accident (CVA), or stroke. CVDs are a very diverse group of disorders that are further classified according to etiology, location, and duration of symptoms. CVDs are subdivided into ischemic events and cerebral hemorrhages, with many etiologies for each. Ischemic events are further classified according to whether symptoms occurred in the carotid or vertebrobasilar distribution and by the duration of symptoms. Transient ischemic attacks (TIAs) seldom last more than a few minutes and never more than 24 hours. In ischemic stroke, the neurologic deficit has been present more than 24 hours and may be progressive, stable, or resolving [6].

About 80% of all cerebrovascular events are ischemic in origin, and most are associated with atherosclerotic disease. The risk of an ischemic event increases with age and is correlated with both systolic and diastolic blood pressure, diabetes, and a history of ischemic heart disease or previous stroke. Ischemic strokes are divided broadly into five categories: large vessel athero-thrombosis, small vessel or lacunar infarction, cardiogenic embolism, other defined causes, and cryptogenic stroke. However, more unusual causes of strokes include vasculitis, drug induced stroke, dissection, or hyper-coagulable states. Hemorrhagic strokes, however, result from the rupture of a blood vessel either into the subarachnoid space or into the parenchymal tissue [6].

Whether a stroke is ischemic or hemorrhagic, appropriate therapy depends upon discernment of its cause, allowing caregivers to prevent progression of the stroke and prevent subsequent strokes. A patient's clinical history is essential to determining the correct diagnosis, for the stroke's time course, progression, and accompanying symptoms greatly inform the investigation. Medical and neurologic examinations provide further insight into a patient's condition and may provide clues to other systemic illnesses that may be contributing factors. Laboratory analyses and neuroimaging have revolutionized our ability to diagnose and treat stroke quickly and accurately, and are areas of intense continuing investigation [5].

For both *ischemic stroke* and *primary hemorrhagic stroke*, the prelude to proper therapy is precise diagnosis that includes not only the extent and location of the infarcted brain, but knowledge of the arterial pathology and the extent of the pared collateral circulation. This is essential to try to devise therapy to prevent further cerebral damage and recurrence of the stroke. In this chapter, cerebrovascular disease is divided into the main primary ischemic and hemorrhagic stroke pathophysiologic. Ischemic cerebrovascular disease is divided into two broad categories: thrombotic and embolic. Cerebral embolic stroke usually occurs abruptly but may present with stuttering, fluctuating symptoms [6].

ANATOMY OF THE CEREBROVASCULAR DISEASE

Anterior Circulation

The brain is supplied anteriorly by paired internal carotid arteries, which provide approximately 80% to 90% of the total cerebral blood flow. The left common carotid artery originates directly from the aortic arch, whereas the right common carotid artery originates from the innominate artery. The common carotid arteries bifurcate at the angle of the mandible into external and internal branches. The external carotid artery has many divisions, several of which supply the cerebral circulation through collaterals. The internal carotid artery can be divided into the cervical [or extracranial], intrapetrous, intracavernous, and supraclinoid segments. The cervical, intrapetrous, and intracavernous portions of the internal carotid artery have no branches. The supraclinoid segment gives rise to the ophthalmic and the posterior communicating arteries before bifurcating into its terminal branches, the anterior and middle cerebral arteries. The intracavernous and supraclinoid segments of the internal carotid artery are referred to as the carotid siphon [7].

Posterior Circulation

The vertebral arteries supply 10% to 20% of the total cerebral circulation. Both vertebral arteries originate from the first portion of their respective subclavian arteries and then enter the vertebral canal at the transverse foramina of the sixth cervical vertebra. The vertebral arteries unite to form the basilar artery, which then branches into the right and left posterior cerebral arteries. The posterior circulation supplies the brainstem, cranial nerves, cerebellum, and the occipital and temporal lobes of the cerebrum [7].

Circle of Willis

The anterior communicating artery connects the two anterior cerebral arteries. The posterior communicating artery connects the internal carotid arteries [anterior circulation] to the posterior cerebral arteries [posterior circulation]. This interconnecting network, which is termed the circle of Willis, is completely intact in 20% to 40% of individuals and allows for collateral flow between the hemispheres and the anterior and posterior circulations [Figure 1].

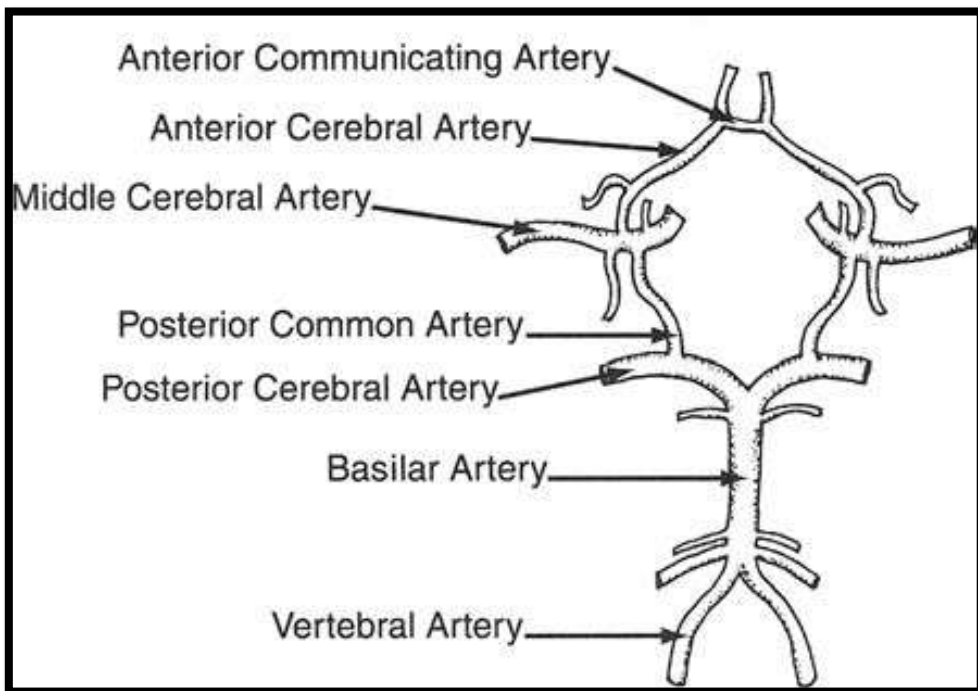


Figure 1.1. Configuration of the terminal branches of the vertebral and internal carotid arteries and their interconnections to form the circle of Willis.

PATHOPHYSIOLOGY OF CEREBROVASCULAR DISEASES

A disruption of arterial flow results in rapid dysfunction of the underlying brain tissue due to two processes: [1] loss of oxygen and glucose necessary for cell processes, and [2]

various alterations to cellular metabolism leading to loss of the cells integrity and cell death. Several changes occur acutely with ischemia: venous blood darkens with decreased oxygen saturation, blood becomes thicker [more viscous], the area of ischemic tissue pales, and arteries narrow. At a molecular level, the normal cellular processes are disrupted (e.g., Krebs cycle) with ATP depletion, increased intracellular calcium and extracellular potassium. Ischemic cells release the excitatory neurotransmitters glutamate and aspartate, leading to an influx of sodium and calcium leading to disruption of the cell membrane and cellular swelling [edema] with cell death [7].

The core region of infarcted tissue is surrounded by a penumbra which represents a zone of hypo-perfused tissue which is vulnerable but may remain viable. The extent of tissue injury is dependent upon the magnitude and duration of the drop in cerebral blood flow which is also dependent upon the support of collateral blood supply from other neighboring arteries. A reduction in cerebral blood flow is often first noted in the farthest territory supplied by that blood vessel. The region representing the boundary of blood flow from two neighboring vessels is termed the border zone, or watershed. Although the core region is irreversibly damaged, the ischemic penumbra has a temporary potential for salvage before progressing to infarction. The focus of current acute ischemic stroke therapy is to restore cerebral blood flow and salvage the penumbra, and stroke research continues to look for a way to extend the timeframe for this to occur by stabilizing the neurochemical milieu or reducing metabolic requirements [7].

Stroke occurs when there is disrupted blood flow to an area of the brain. The brain is perfused by anterior and posterior circulations. The anterior circulation begins with the carotid arteries and perfuses the anterior four fifths of the brain. The internal carotid arteries give off the ophthalmic branch before terminating at the circle of Willis, branching into the anterior and middle cerebral arteries. The posterior circulation supplies only one fifth of the brain and is derived from the vertebral arteries. The vertebral arteries first supply the cerebellum via the posterior inferior cerebellar arteries. The vertebral arteries then join to form the basilar artery. The basilar artery branches to form the posterior cerebral arteries that supply the occipital lobes. Arterial and cardiac abnormalities [8] (see Figure 1.2) often develop and lead to ischemic stroke. The most common cause of ischemic stroke is atherosclerosis of large and small arteries.

When atherosclerosis of an arterial wall leads to thrombus formation, there is partial or complete disruption of blood flow (thrombotic stroke). The thrombus may also dislodge and occlude a more distal artery [embolic stroke]. Large artery pathology commonly occurs in the aortic arch, carotids, and major cerebral vessels. Small vessel atherosclerosis may involve intracranial or penetrating arteries, with more focal or limited brain injury occurring (lacunar stroke). The most common cardiac abnormalities leading to stroke are atrial fibrillation, valvular disease [especially mitral stenosis], and poor ejection fraction. These cardiac problems predispose to thrombus formation, with dislodged thrombi [emboli] occluding cerebral vessels. Less common causes of ischemic stroke include drug use, arterial dissection, fibromuscular dysplasia, arteritis, hypercoagulable states, and migraines [8].

Hemorrhagic strokes comprise approximately 20% of total strokes and are divided into intracerebral and subarachnoid types. Intracerebral hemorrhage is usually caused by hypertensive vascular disease, with bleeding most often into the putamen, thalamus, pons, or cerebellum. Subarachnoid hemorrhage (SAH) is typically the result of leaking from a saccular aneurysm, but may be secondary to an arteriovenous malformation or other vascular

abnormality. A wide spectrum of pathology including atherosclerosis, aneurysmal disease, dysplasias, and arteriopathies can affect the cerebrovascular circulation. Atherosclerosis of the carotid bifurcation, however, is the most common lesion that produces symptoms. Atherosclerosis is the pathological process most often responsible for cerebrovascular insufficiency. The carotid bifurcation is the predominant location for atherosclerotic disease [9].

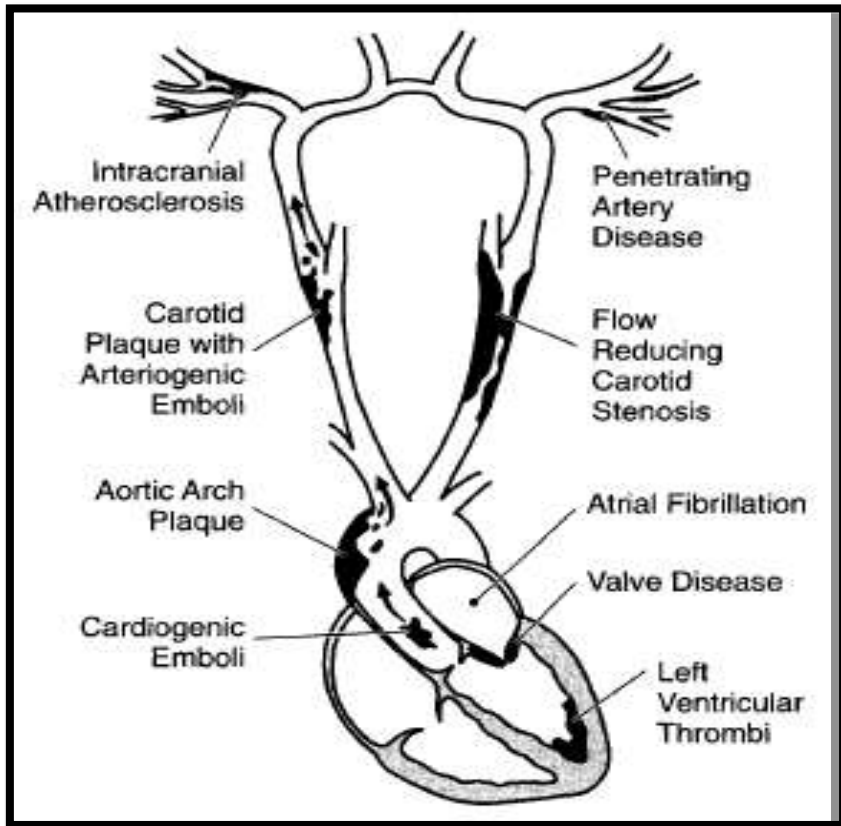


Figure 1.2. The most frequent sites of arterial and cardiac abnormalities causing ischemic stroke. [From Albers EW, et al.]

Low shear stress in well-defined regions of the carotid bulb appears to stimulate the formation of atherosclerotic plaque. Symptoms of cerebrovascular disease may be the consequence of distal embolization from an atherosclerotic plaque or hypoperfusion related to a flow-limiting lesion. The most common cause of a cerebral ischemic event, however, is embolization. Platelets accumulate on the thrombogenic surface of an irregular or ulcerated plaque. Symptoms are produced when these platelet and cholesterol aggregates embolize and obstruct the more distal circulation. Plaque hemorrhage with rupture and extrusion of thrombus into the arterial lumen is another mechanism by which embolization can occur. Although an ulcerated atherosclerotic lesion can embolize even if there is no luminal

encroachment, highly stenotic plaques are more often ulcerated and thus more likely to produce emboli [10].

Hypoperfusion related to carotid artery stenosis is a less common source of symptoms because extensive collateral circulation is provided by the contralateral carotid and vertebral arteries via the circle of Willis and by the external carotid artery via transcranial connections. The fact that 90% to 95% of patients undergoing CEA do not develop cerebral insufficiency during clamping of the carotid artery confirms that, in the majority of individuals with progressive atherosclerotic disease, this collateral network is adequate to prevent cerebral ischemia [11].

Atherosclerosis commonly involves the large extracranial vessels that arise from the aortic arch. Although the carotid bifurcations are most frequently involved, atherosclerosis can also occur at the origins of the common carotid or vertebral arteries or in the intracranial vessels, including the carotid siphon and the basilar artery. Atherosclerosis probably results in ischemic symptoms through several mechanisms. The most widely accepted is platelet activation and aggregation at the site of an ulcerated complex atherosclerotic plaque, with production of thromboxane A_2 from arachnidonic acid resulting in further platelet aggregation. Aggregated platelets can embolize, with specific symptoms dependent on the vessel of embolization. The severity of symptoms depends on the duration of vessel occlusion and the degree of collateral flow through tiny leptomeningeal and other end artery anastomoses [5]. Atherosclerosis may also result in vessel stenosis or occlusion. In this situation, symptoms depend largely on how rapidly stenosis develops and the extent of collateral flow available through the circle of Willis and from extracranial–intracranial anastomoses. The availability of collaterals varies considerably among different people; thus, the same degree of stenosis or occlusion can result in very different symptoms. For example, total occlusion of the internal carotid artery may be asymptomatic in one individual, but result in a disastrous stroke in a patient with congenital absence of portions of the circle of Willis. Other factors that are probably important in determining outcome are blood viscosity, blood glucose, blood oxygen carrying ability, and tissue metabolic demand [13-14].

Stenosis of small intracranial arteries can also result in ischemic symptoms. Patients with hypertension or diabetes can develop atherosclerosis of small intracranial arteries. A more common occurrence is the development of hypertension-related lipohyalinosis and fibrinoid necrosis in small end arteries and arterioles. When this results in occlusion, there is no available collateral flow, and a tiny "lacunar stroke" results. Among the many lacunar syndromes are pure motor hemiparesis with or without dysarthria [due to lesions of the internal capsule or pons] and pure sensory stroke [due to lesions in the thalamus]. Patients with lacunar strokes almost never have field defects, aphasia, or other higher cortical function loss [15].

Emboli from the heart cause 10 to 20% of ischemic strokes. Although many cardiac conditions predispose to cerebral embolization, the two most common are atrial fibrillation and acute myocardial infarction. The risk of stroke is increased five times in patients with atrial fibrillation and fifteen times if there is associated mitral stenosis. Strokes complicate acute myocardial infarctions approximately 2% of the time. The risk is highest in patients with large infarctions, those with congestive heart failure, or those with anterior infarctions where there is hypokinesis of the left ventricular apex. Emboli usually lodge in end arteries that have poor collateral circulation and therefore often cause major neurologic deficits. The middle cerebral artery distribution is usually affected [5-6].

CARDIAC CONDITIONS ASSOCIATED WITH EMBOLIZATION

Other rare causes of ischemic stroke include hematologic disorders [polycythemia, thrombocytosis, dysproteinemias, sickle cell disease], fibromuscular dysplasia, carotid dissections, and intracranial vasculitis of several etiologies [lupus erythematosus, giant cell arteritis, syphilitic arteritis, granulomatous angiitis]. Clinical clues and past history will usually help identify these unusual conditions [5].

The development of saccular aneurysms of the circle of Willis is thought to occur gradually at bifurcation sites where the arterial media may be congenitally absent. The internal elastic lamina at these locations becomes fragmented, possibly accelerated by atherosclerosis. Aneurysms have a predilection for certain locations, especially the posterior communicating artery, anterior communicating artery, and middle cerebral artery. They are multiple in 20% of cases. Progressive enlargement seldom causes symptoms unless the aneurysm compresses an adjacent structure, for example the oculomotor nerve by a posterior communicating artery aneurysm [16-18]. Because cerebrovascular disorders are a diverse group of illnesses that are managed in very different ways, an accurate diagnosis is critical. The following points are important for proper diagnosis and management [19]: [See Table 1.1]

Table 1.1. Important points regarding diagnosis and management of strokes

S. No	Important points for diagnosis and management of strokes
1	Establish that a cerebrovascular event actually occurred, and determine the location of the event.
2	Decide if the event was ischemic or hemorrhagic.
3	Determine what steps are necessary for medical stabilization of the patient.
4	Determine the underlying pathophysiology that caused the event (e.g., atherosclerosis, embolus, aneurysm).
5	Determine what can be done to prevent future, possibly more devastating, events.
6	Decide if the occurrence of the cerebrovascular event may indicate an underlying disease (especially cardiac diseases).
7	When a fixed deficit already exists, establish the patient's potential for rehabilitation.

Differentiating between ischemic stroke and intracranial hemorrhage is usually easy because the latter is often much more catastrophic with precipitous onset of severe headache, nausea and vomiting, and clouding of consciousness. Hemorrhages of all etiologies tend to occur in younger patients than those afflicted by ischemic stroke. Although it is often said that

ischemic strokes occur at rest or during sleep, whereas hemorrhages occur during straining or exertion, this is not always a reliable rule. Although headaches are more common in hemorrhage, they can also occur in ischemic stroke, and not every patient with a hemorrhage has a headache. Vomiting within 15 minutes of onset is highly suggestive of hemorrhage. Small intracerebral hematomas may be indistinguishable from ischemic stroke. The differentiation is readily made by CT scan, which has become almost routine in evaluation of patients with stroke and is mandatory if anticoagulation is contemplated [20]. With long-term neurologic deficits due to cerebrovascular disease, it is important to assess the patient's ability for functional living. The quality of life of many patients is improved considerably by participation in a comprehensive rehabilitation program.

SUB-TYPES OF STROKE AND THEIR CATEGORIZATION

The two main categories of cerebrovascular disease are ischemic and hemorrhagic. Ischemic stroke is due to a lack of blood flow to part of the brain. Occlusion of a cerebral artery by a blood clot that travels from the heart or another vessel (embolus) or that develops within a cerebral artery [thrombus] results in an arterial ischemic stroke. Diminished cerebral blood flow due to narrowing of a blood vessel or decreased blood pressure also may result in ischemic brain injury. Less commonly, a blood clot develops within one or more veins that drain the brain, known as cerebral venous sinus thrombosis, and leads to venous infarction. Hemorrhagic stroke occurs when a blood vessel ruptures, leading to brain injury

As per pathophysiology strokes can be divided into three main categories like Ischemic, hemorrhagic and cerebral venous thrombosis (see Table 1.2 and Figure 1.3). The major categorization of stroke is whether it is *Ischemic* or *Hemorrhagic*. The archaic term “cerebrovascular accident” should not be used. *Ischemic stroke* (IS) or *cerebral infarction* occurs when an arterial flow to the brain is obstructed and accounts for ~85% of all strokes. *Hemorrhagic stroke* occurs when an artery or vein ruptures causing intracranial bleeding and accounts for ~15% of all strokes.

Hemorrhagic stroke occurs due to a blood vessel rupture in the brain. Its harmful effects are a resultant of: [a] hypoxia due to disrupted vascular supply; [b] irritant effect of releasing blood on brain parenchyma and vasculature; and [c] raised ICP due to continued bleeding, which may further restrict cerebral blood flow. In this respect, hemorrhagic strokes are more dangerous than ischemic strokes. There are two types of hemorrhagic stroke: intra-cerebral hemorrhage [generally occurs in small arteries or arterioles and is commonly due to hypertension, trauma, bleeding disorders, amyloid angiopathy, illicit drug use like amphetamines or cocaine, and vascular malformations], and sub-arachnoid hemorrhage [due to rupture of aneurysms from the base of the brain and bleeding from vascular malformations near the pial surface]. It constitutes only 10–15% of all strokes [21].

Table 1.2. Sub-tyapes of stoke and their causes

Ischemic Stroke or Infraction	Hemorrhagic strokes	Cerebral venous thrombosis
A. Large artery atherosclerosis	A. Intracerebral hemorrhage 1. Hypertension 2. Amyloid angiopathy 3. Arteriovenous Malformation (AVM) 4. Hematologic disorder 5. Hemorrhagic transformation of recent cerebral infarct 6. Hemorrhage into tumor	A. Dural sinus thrombosis
B. Lacunar or small vessel arteriosclerosis		
C. Cardioembolism		
D. Cryptogenic	B. Subarachnoid hemorrhage 1. Saccular aneurysm 2. Arteriovenous malformation 3. Hematologic disorder 4. Mycotic (infectious) aneurysm	B. Cortical venous thrombosis
E. Other known cause 1. Cervicocephalic dissection 2. Coagulopathy 3. Non-atherosclerotic arteriopathy (e.g., CADASIL) 4. Metabolic disorders (e.g., MELAS) 5. Migraine 6. Vasculitis 7. Drug abuse		

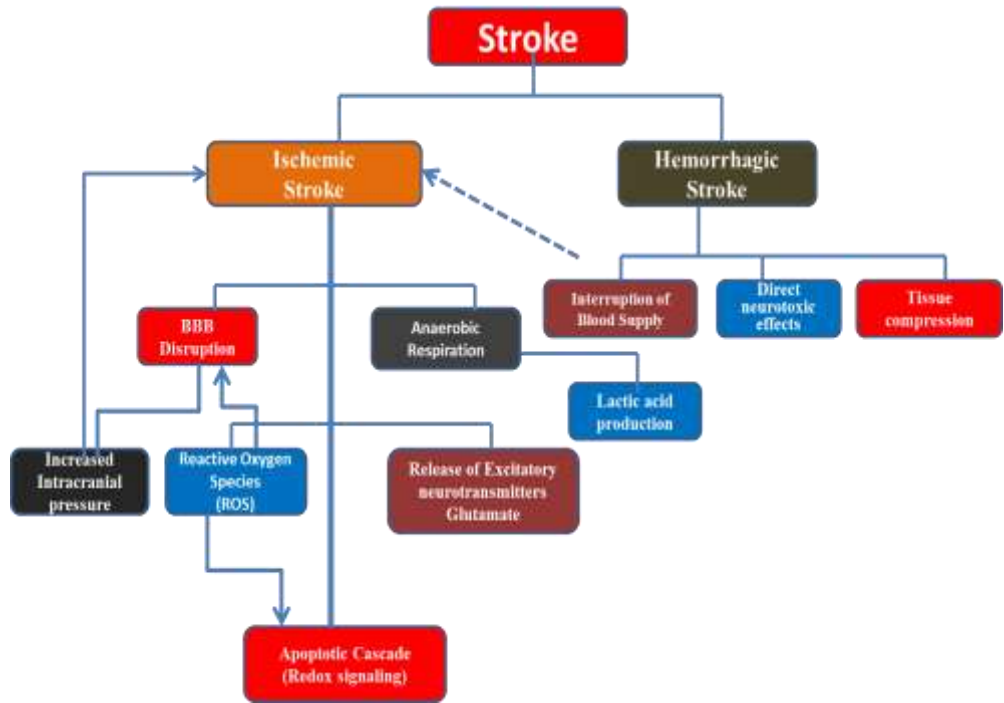


Figure 1.3. Schematic showing different types of stroke and cascade of events following ischemia.

ATHEROSCLEROSIS AND STROKE

Atherogenesis is a decade-long process which involves luminal obstruction by cellular and extracellular substances. The pathogenetic process from onset of atherosclerotic changes in cerebrovascular or extracranial circulation to precipitation of acute ischemic stroke with its consequent cell damage is complex and many of the intermediary steps are not fully understood. Changes may manifest in the form of: [a] *fatty streak*, earliest lesions seen as yellowish areas of discoloration of intima, due to accumulation of lipid-filled macrophages [foam cells] in approximately 30% children below 5 years [22]; [b] *more advanced lesions with massive extracellular lipid* at the branching points of arterial vessels, in late childhood and early adolescence [23]; [c] *complicated fibrous plaques*: central acellular area of lipid covered by a cap of smooth muscle cells and collagen, seen in the third decade of life [23]. Persons with risk factors for atherosclerotic disease (e.g., hypertension, hypercholesterolemia, cigarette smoking) tend to have clinically advanced atherosclerotic lesions with increased frequency. Sequence of events in atherogenesis is:

1. Injury to Arterial Wall

In his “response-to-injury” theory, Ross had hypothesized that, atherosclerosis is the effect of the complex interplay among monocytes, lipoproteins, platelets, lymphocytes, and smooth muscle cells in the intimal layer [24]. Three-tier pathophysiologic classification system of injury to arterial wall was put forth by Ip et al. in 1990 [23].

- *Type I injury*: Chronic minimal injury with functional alterations of endothelial cells without significant morphological changes, primarily caused by the turbulence of blood flow. Other contributory factors being hypertension, hypercholesterolemia, circulating vasoactive amines, immunocomplexes, viral infections, and tobacco smoke.
- *Type II injury*: Endothelial denuding and superficial internal injury, possibly due to toxic products released by accumulating macrophages in the intima. These changes may be accompanied by platelet deposition with or without thrombus formation, and subsequent thrombus incorporation.
- *Type III injury*: Manifested by deep intimal and medial damage, accompanied by marked platelet aggregation and mural thrombosis, generally following plaque rupture.

2. Role of Monocytes and T-Lymphocytes in Foam Cell Transformation

The next important events are:

- *Circulating monocyte adhesion*: Abnormal shear stress at atherosclerotic lesion-prone sites in the circulatory system enhances the production of certain transcription factors, which promotes the expression of endothelial vascular cell adhesion

molecule [VCAM], which is imperative for monocyte binding to endothelial cells [25].

- *Monocytes insinuation* between the tight junctions of the endothelial cells to enter the subendothelial space.
- *Activation of immune mechanism*: In the form of T-lymphocyte activation, in both early fatty lesions and in advanced fibrous lesions, which helps monocytes migration and its transformation into “foam cell”.

3. Oxidation of LDL-Cholesterol

Oxidation of LDL cholesterol induced by free radicals produced by macrophages, endothelial cells, or smooth muscle cells, participates by:

- a) Formation of foam cells;
- b) Cytotoxic properties promoting endothelial injury;
- c) Chemoattractant for circulating monocytes; and
- d) Inhibiting egress of macrophages from plaques [26].

4. Smooth Muscle Cell Migration and Proliferation

A number of molecular factors may play a role in this vital plaque-forming process. They include growth factors [e.g., platelet-derived growth factor, or PDGF, a polypeptide released from blood platelets and endothelial cells that may attract smooth muscle cells to the intima and encourage them to divide]; eicosanoids [which can stimulate the hydrolysis of cholesterol ester, producing free cholesterol]; certain cytokines [e.g., tumor necrosis factor, interleukin-1 and interferon]; and nitric oxide which acts to dilate blood vessels.

5. Role of Platelet

Platelet aggregation and adhesion promoted by toxic products released by macrophages and by moderate damage to the internal surface with denudation of the epithelium have vital roles in the progression of atherosclerosis [23].

6. Plaque Fissuring and Thrombus Formation

The process of plaque destabilization [fissuring and rupture, followed by thrombus formation] is not fully understood.

- **Plaque fissuring is possibly due to:**
 - a) Loss of an internal lattice of collagen supporting the cap of the plaque., making it vulnerable to circumferential stress during systole;

- b) Infiltration of the cap tissue with foam cells, which possibly weakens the tissue by passively distorting the spatial arrangement of the connective tissue matrix or by actively destroying connective tissue matrix protein by lytic mechanisms [27].
- **Thrombus formation occurs by:**
 - **Platelet activation:** On coming in contact with the sub-endothelial collagen, exposed by way of plaque rupture, circulating platelets get activated, and subsequently aggregate and adhere, through interaction with platelet surface receptors [most important being GP-Ib-IX] and sub-endothelial protein ligand von Willebrand factor [VWF]. An association between elevated vWF concentrations and arterial thrombosis has been described [28].
 - **Platelet activation and blood flow:** Under high fluid shear-stress conditions, as in atherosclerosis, there is evidence that platelet aggregation depends on the binding of VWF to platelet GPIIb/IIIa, blockade, of which inhibits platelet aggregation and thrombus formation without disturbing the initial platelet adhesion [29 Stoll G 2008].
 - **Activation of the coagulation cascade:** Endothelial injury constitutes the primary impetus for the initiation of the extrinsic pathway of the coagulation cascade. Activated platelets, apart from forming the backdrop of the clotting process, also acquire enhanced capacity to catalyze interactions between activated coagulation factors [which normally circulate as inactive precursors: *zymogens*]. Factor Xa, is the active catalytic component of the “prothrombinase” complex, which converts prothrombin to thrombin. Thrombin cleaves fibrinopeptides [FPA, FPB] from fibrinogen, allowing the resultant fibrin monomers to polymerize, and converts Factor XIII to XIIIa, which crosslinks [XL] the fibrin clot. Thrombin accelerates the process by its potential to activate Factors V and VIII, while a number of natural plasma inhibitors retard clotting, including C1-inhibitor [C1 INH], tissue factor pathway inhibitor [TFPI], and antithrombin II [ATIII]. The fibrin molecules aggregate together, trapping platelets, erythrocytes, and leukocytes to form the thrombus. Subsequently, there is a tendency for the clot to enlarge as blood flow slows around it, resulting in enlargement of size [*“propagating thrombus”*].
 - **Physiologic-subtypes of thrombosis related ischemic stroke:**
Ischemic stroke with different etiologies, possibly have a link based on the process of thrombosis:
 - Atherothrombotic occlusion of larger arteries [e.g., carotid, middle cerebral, basilar]: Most common causes of primary large vessel occlusive cerebrovascular disease, and also is the most common cause of stroke.
 - Embolism is the second most common cause of stroke. Most are due to cerebral arterial atherothrombosis; or may also arise from other cardiogenic sources and deep vein thrombosis.
 - Microatheroma of an influx of fat-like materials [lipohyalinosis] affects small vessels; and most prominently causing lacunar strokes.

7. The Potential Outcomes of Plaque Fissuring

It includes

- a) Sealing of the fissure with fibrosis of incorporating thrombus; or
- b) Mural intra-intimal and intra-luminal thrombosis, resulting in partial or transient reduction in blood flow [precipitating transient ischemic attacks: TIA]. It may progress to occlusive thrombosis, which, if persistent due to absence of collateral flow, can lead to ischemic stroke [23 IP JH 1990].

8. Evolution of Cerebral Atherothrombosis

Thrombosis may evolve over a few minutes, or take hours or even days. A stroke that is actively progressing as a direct result of increasing occlusion and ischemia is termed '*stroke in evolution*' or '*progressing stroke*'. A large blood vessel may evolve over a longer period of time, as compared to a smaller vessel, and there may be warning signs like TIA. Damage in ischemic stroke may result not only from infection, but also from edema, which generally peaks in 2–5 days. [I] *Hemorrhagic conversion*: Natural consequence of reperfusion of blood through damaged blood–brain barrier, in ischemic stroke, generally has 'bland' infarction associated with secondary bleeding. This is referred to as hemorrhagic conversion or hemorrhagic transformation [HT], and is characterized by gross parenchymal hematoma with possible intraventricular extension, midline shift and herniation; along with widespread leukocyte infiltration and macrophage invasion [30]. HT may be seen as hemorrhagic infarction [HI] or, less commonly, parenchymatous infarction [PH]. Though both have different incidences, pathogenesis, and clinical outcome, but distinguishing HI and PH on CT may be difficult. On CT, HI appears as a discontinuous heterogeneous mixture of high and low densities occurring within the vascular territory of the infarct. In contrast, PH appears as a discrete, homogeneous collection of blood that often exerts mass effect and may extend beyond the original infarct boundaries or even into the ventricles [30].

Recent studies have suggested the role of an abnormal expression of some matrix metalloproteinases [MMPs] which can degrade almost all components of the extracellular matrix and basal lamina such as laminins, fibronectin, or type IV collagen, weakening brain microvessels and predisposing them to rupture and increasing the risk of cerebral hemorrhage and precipitate hemorrhagic transformation events after stroke [31]. Previous animal models [32–33] have demonstrated an abnormal expression of MMP-2 [Galatians A] or MMP-9 [Galatians B] after cerebral ischemia and in lipopolysaccharide-injured brains [34], contributing to brain injury and BBB breakdown. Further, Asahi et al. 2001 and Sumii et al. 2002 [35–36] reported that pharmacological or genetic inhibition of MMP-9 has caused a significant reduction in size of the infarct, as well as the risk of hemorrhagic complications, while Wang et al. 2003 [37] and Tsuji et al. 2005 noted tissue plasminogen activator-associated hemorrhage and edema appeared to be correlated to MMP-9 dysregulation. Investigations dealing with ischemic stroke in human subset have revealed high MMP-9 levels in peripheral blood, with MMP-9 levels showing correlation with poor neurological outcome, infarct growth, and hemorrhagic transformation events [38–39].

Rossel et al. 2008 [40] have reported a strong neutrophil infiltration in the infarcted and hemorrhagic areas with local high MMP-9 content closely related to basal lamina collagen IV degradation and blood–brain barrier breakdown. Hemorrhagic infarction [HI] occurs regularly in the natural evolution of acute embolic stroke and is usually asymptomatic. Autopsy studies of HI may vary from patchy petechial bleeding to more confluent hemorrhages, with an occurrence rate ranging from 51% to 71% of recent embolic strokes [30]. According to another estimate [41], approximately 20% of patients with cardioembolic stroke have hemorrhagic transformation in the infarcted zone, usually occurring within 48 h, and is rare in the first 6 h. HI has been often explained as a result of reperfusion of the vascular bed of the infarct, such as would occur after fragmentation and distal migration of an embolus or after early reopening of a large vessel occlusion in the setting of a large infarction; the full pressure of arterial blood into hypoxic capillaries results in a diapedesis of red cells through their hypoxic walls [42]. It has been observed that HI may develop in areas distal to a persisting occlusion, suggesting that reperfusion is not always a prerequisite for this phenomenon. Many studies have found strong correlation with risk factors like:

- 1) One or more surges of arterial hypertension;**
- 2) Associated hyperglycemia; and**
- 3) Restoration of blood flow to ischemic territories.**

Possible mechanism for this is marked tissue energy depletion accompanied by acidosis damaging brain vessels, causing leakage of edema fluid and red blood cells.

Parenchymatous infarctions (PH) occur less frequently, but are often symptomatic due to extension and mass effect beyond the original infarct territory [30]. In contrast to HI, it has been proposed that the pathogenesis of PH may involve “ischemic necrosis resulting in the rupture of small penetrating vessels analogous to hypertensive hemorrhage, leading to massive bleeding”. Trials of thrombolytic therapy for acute ischemic stroke, has revived interest in this, since PH appears to be associated with anticoagulation therapy [26, 43].

Treatment approaches for ischemic stroke and cerebrovascular diseases have largely focused on reperfusion of the ischemic brain tissue—for example with the thrombolytic agent recombinant tissue plasminogen activator [rt-PA, The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group, 1995]—and on neuronal protection, mainly from damage caused by excitotoxic amino acids such as glutamate. Over the past 15 years it has been increasingly appreciated that inflammation alone, or in combination with systemic immune system responses, may be an important contributing factor in stroke-related brain injury and stroke outcome, as well as in the development of atherosclerotic cerebrovascular disease. Elements of the inflammatory process may provide promising targets for the treatment of acute ischemic stroke and for vascular disease prevention.

The role of inflammation in atherosclerotic cerebrovascular disease has also been increasingly recognized to the effect that atherosclerosis is no longer regarded as a disorder of lipid storage within blood vessels but now, primarily, as a disorder of chronic inflammation [44]. These changing concepts of the role of the immune system and inflammation in stroke and cerebrovascular disease have led to searches for new treatment and prevention strategies. Here we discuss the current state of knowledge about inflammation and the immune system in stroke and atherosclerosis and discuss potential therapeutic strategies.

ATHEROSCLEROTIC CEREBROVASCULAR DISEASE AND INFLAMMATION

The study of cerebrovascular disease has advanced markedly in recent years with advances in non-invasive imaging methods such as MR angiography and CT angiography as well as an improved understanding of the immune system in the pathogenesis of atherosclerosis. Atherosclerotic cerebrovascular disease is a common cause of strokes and shows a predilection for sites such as the bifurcation of the common carotid artery into the internal and external carotid arteries and the aortic arch and the major intracranial arteries such as the basilar artery and the middle cerebral arteries. Occlusive atherosclerotic vascular disease of these large extracranial arteries is responsible for as many as 20–30% of ischemic strokes and intracranial steno-occlusive disease causes around 5–10% of ischemic strokes.

Atherosclerosis is characterized by the formation of plaques within the innermost layer of artery walls. It has traditionally been regarded as a disorder of lipid storage but is now regarded as a chronic inflammatory disorder. The plaque commences with the deposition of cholesterol-containing lipoproteins in the vessel wall. The first inflammatory cells involved are believed to be circulating monocytes [rather than neutrophil polymorphs]. These migrate across the endothelial lining from the blood or from the lymphatics and develop into tissue macrophages or into dendritic cells. The macrophages [“foam cells”] accumulate cholesterol in the artery wall. The resulting fatty streaks commence as early as the late teen years or the early 20s and may regress or progress throughout life. Lesions are typically asymptomatic, at least initially. Many fatty streaks develop into atheromatous plaques that contain extracellular debris and cholesterol. A fibrous cap is formed over the lipid-containing plaque by smooth muscle cells from the arterial wall and possibly from blood-derived progenitor cells. The collagen stabilizes the lesion and prevents debris, lipids and pro-coagulant material from reaching the bloodstream [45].

Pro-inflammatory lymphocytes [i.e., CD4⁺ Th1 cells] also contribute to and may accelerate atherosclerotic plaque formation [44]. CD4⁺ CD28[–] cells [mentioned previously] are a rare subset of CD4⁺ T cells that are long-lived, secrete high levels of γ -IFN and are directly cytotoxic. These cells preferentially infiltrate unstable plaques and contribute to increased endothelial cell lysis in patients with acute coronary syndromes [46]. Naturally arising regulatory T cells [CD4⁺ CD25⁺] have recently been shown to be potent inhibitors of the development of atherosclerosis in several mice models and may be a promising target for vascular disease prevention [47]. It has also been suggested that common infections may be associated in the pathogenesis of atherosclerosis. Specific organisms that have been studied include *Chlamydia pneumoniae*, herpes viruses, *human immunodeficiency virus*, *Helicobacter pylori*, and organisms associated with periodontal infections; however definitive proof of causal associations are lacking.

The atheromatous plaque grows silently for years or even decades without producing clinical symptoms. However, trace amounts of pro-inflammatory cytokines released from cells within the plaque, including IL-6, reach the bloodstream and stimulate C-reactive protein [CRP] secretion by the liver. CRP can be detected in the peripheral blood and is an independent marker of risk for myocardial infarction and probably for stroke in healthy individuals. Atherosclerotic plaques precipitate ischemic strokes by rupturing, leading to superimposed thrombus formation and embolism. They may also lead to strokes if the

narrowing becomes particularly severe, >50%. The risk of stroke is greater with the greater degrees of narrowing as there is also associated hemodynamic insufficiency. Conventional angiographic techniques and non-invasive modalities [ultrasound, MRA, CTA] are used to detect occlusive arterial disease, and in the case of stroke prevention a narrowing of $\geq 50\%$ of the internal carotid artery is used in decision-making for referral of patients for carotid endarterectomy.

Certain plaques are particularly prone to rupture. These “vulnerable plaques” contain abundant inflammatory cells and have thin collagen caps. The atheroma’s vulnerability to rupture is correlated with higher cellular infiltrates of macrophages and activated T cells. In symptomatic carotid artery plaques elevated ICAM-1 expression [relative to asymptomatic plaques] has been found and the ICAM-1 expression was greatest in the high-grade region of the plaque. Vulnerable plaques are also not necessarily the largest plaques, particularly in patients with coronary artery disease, and current angiographic methods are of limited value in their recognition. However, new MRI methods using novel contrast agents are now being used to better study the features of vulnerable plaques.

RISK FACTORS FOR STROKE

As mentioned above, atherosclerotic vascular disease is a common cause of ischemic stroke and also predisposes patients to coronary artery disease, itself a risk factor for stroke. Vascular disease also increases with age and is increased in patients with hypertension, diabetes, a smoking history and hyperlipidemia. Hypertension is the major risk factor for hemorrhagic stroke. The “metabolic syndrome” is a cluster of risk factors [truncal obesity, hyperglycemia, hypertriglyceridemia, hypertension and low HDL-cholesterol] associated with increased cardiovascular disease risk. Some immune and inflammatory risk factors may include recent infection that increases the risk of stroke to a modest degree and the possible mechanisms for this are being investigated. Periodontal disease, another factor shown to be independently associated with stroke and coronary artery disease, is believed to be associated with chronic inflammation and endothelial dysfunction [48]. Lipoprotein associated phospholipase A2 (a pro-inflammatory enzyme associated with low density lipoprotein) is approved by the Food and Drug Administration as a risk marker for stroke and coronary artery disease. Other inflammatory markers in the blood are showing promise as independent risk factors; these include elevated plasma levels of high sensitivity-CRP, IL-6 and the total white blood cell count [49].

MIXED CEREBROVASCULAR DISEASE

The concept of “mixed cerebrovascular disease” has been proposed as a framework for better understanding stroke, and for improving stroke prevention efforts [50]. Mixed cerebrovascular disease incorporates clinical and subclinical stroke with hemorrhagic and ischemic stroke. The clinical stroke syndromes thus incorporate the typical variety of presentations encountered, with intracerebral hemorrhage and ischemic stroke subsets (small

vessel disease, large vessel disease, cardiogenic, other). Subclinical stroke syndromes include silent ischemic stroke and cerebral microbleeds.

Cerebral white matter disease (of aging) is an additional component of mixed cerebrovascular disease. There are both strengths and weaknesses of this conceptual approach. A principal benefit of characterizing a given patient as having “mixed cerebrovascular disease” is that the clinician is immediately confronted with the fact that, going forward with stroke prevention efforts, this patient will require a complex strategy. Simply relying on “antiplatelet medications” for prevention of cerebral infarction will be insufficient. The principal weakness of this approach is that it may simply be too inclusive. For example, cerebral white matter changes are ubiquitous in the population of age 65 and older, with more than 95% of individuals showing at least some white matter change on magnetic resonance imaging (MRI) [51]. Only about one-third of these changes are probably sufficient to be characterized as “disease”, but the gradation between normal and pathological change remains unclear.

A similar problem may arise with the inclusion of cerebral microbleeds. Microbleeds are present in approximately 20% of the population beginning at age 60, a proportion that increases to nearly 40% by age 80 [52]. Pathological studies reveal a much higher prevalence over the age of 70 [53-54], but it is unclear whether both MRI and neuropathological findings are demonstrating the same entity. However, this difficulty may be surmounted by simply focusing on the inclusion of MRI-demonstrable cerebral microbleeds, using gradient echo or the more sensitive susceptibilityweighted imaging sequences.

To summarize, the use of mixed cerebrovascular disease as a conceptual and clinical framework appears feasible. Inclusion of cerebral white matter disease may be problematic. Nevertheless, a characterization of stroke syndromes that incorporates clinical and subclinical ischemic and hemorrhagic disease may help quickly characterize the complexity of many stroke patients.

Mixed cerebrovascular disease represents the Scylla and Charybdis of modern stroke neurology. The stroke neurologist must navigate between the apparent extremes of ischemic and hemorrhagic processes. For the most part, the patient will come to the attention of the neurologist with symptoms suggesting ischemic stroke, and cerebral microbleeds and white matter disease will be identified incidentally. Given its relationship to hemorrhagic stroke, the presence of cerebral microbleeds is particularly challenging. An attractive therapeutic strategy for mixed cerebrovascular disease is one that targets both the coagulation system and the vessel wall. Platelet agents may be focused on receptor antagonism, but inhibition of signal transduction pathways is an important alternative strategy for inhibition of platelet activation pathways. Inhibition of platelet signal transduction can be achieved by manipulation of platelet second messenger pathways and/or amplification of effects of endothelial-derived molecules [e.g., prostacyclin and nitric oxide] that activate cyclases producing elevated levels of intracellular cyclic adenosine monophosphate [cAMP] and cyclic guanosine monophosphate (cGMP) [55]. Importantly, cAMP pathways have well-described major roles in development of the blood–brain barrier [56]. Platelet levels of cyclic nucleotides have critical regulatory function, so that elevated levels of cAMP and cGMP interfere with all known platelet activation pathways [55].

Signaling of cyclic nucleotides is modulated by their hydrolysis by phosphodiesterases (PDEs), with the latter regulated by any of the more than 60 isoforms of the eleven families of PDE inhibitors [55]. Notably, the PDE inhibitors dipyridamole and cilostazol have been

shown to have beneficial actions for ischemic stroke prevention [57-60]. However, neither drug has been considered a first-line agent for stroke prevention efforts. Dipyridamole, a relatively nonspecific PDE inhibitor with effects on both PDE3 and PDE5 [55], has been shown beneficial for the prevention of ischemic stroke, with stroke risk reduction comparable to that seen with aspirin [57]. Dipyridamole is known to have dual actions, combining platelet anti-aggregant effects and vessel wall protection [61]. Platelet actions are generated by adenosine-mediated effects, along with potentiation of the platelet anti-aggregatory effects of prostacyclin and nitric oxide [61]. Dipyridamole inhibits red blood cell uptake of adenosine, resulting in elevated plasma adenosine leading to stimulation of platelet adenylyl cyclase and increased platelet cAMP [57, 61]. Vessel wall protection effects of dipyridamole are produced via anti-oxidative effects and by inhibition of platelet-monocyte interactions [61].

Recent work with animals has emphasized the potential for dipyridamole in stroke prevention as well as treatment. In summary, an attractive therapeutic strategy for mixed cerebrovascular disease is one that utilizes agents acting on both platelets and vessel wall. Several PDE inhibitors have those dual actions. Dipyridamole and cilostazol have already been shown effective in ischemic stroke prevention clinical trials and may be particularly useful for patients with mixed cerebrovascular disease.

Mixed cerebrovascular disease consists of ischemic and hemorrhagic phenomena, both clinically evident diseases as well as subclinical processes. Ischemic stroke, subclinical infarct, and white matter disease of aging (or leukoaraiosis) combined with intracerebral hemorrhage and cerebral microbleeds constitute this entity. The incorporation of these processes into a single entity creates a novel concept in stroke diagnostics. The treatment of mixed cerebrovascular disease presents the stroke clinician with a profound dilemma. What is the way out of this conundrum? Use of PDE inhibitors, combining platelet and vessel wall effects, provides one possible strategy. There are, no doubt, other approaches that will become increasingly obvious in the coming decades.

In adults, arterial ischemic stroke is commonly associated with advancing age, hypertension, atrial fibrillation, smoking, and diabetes mellitus [62]. Other risk factors include obesity, cardiac disease, carotid stenosis, sickle cell anemia, recent infection, and alcohol abuse. In young adults abnormalities of blood vessel structure such as arterial dissection, non-inflammatory vasculopathies, and vasculitis are also associated with stroke [63]. In addition, hematologic abnormalities leading to hypercoagulability may play a role in selected cases [64]. Cerebral venous sinus thrombosis, which can result in either ischemic or hemorrhagic infarction, is associated with oral contraceptive use; infections of the head, neck, or central nervous system; malignancy; prothrombotic states; inflammation; and pregnancy [65]. In fact, the risk of both ischemic and hemorrhagic stroke is increased during pregnancy and the post-partum period [66].

A common risk factor for primary intracerebral hemorrhage in adults is hypertension. Other risk factors include amyloid angiopathy, elevated cholesterol, treatment with anticoagulants, heavy alcohol use, smoking, renal dialysis, and use of sympathomimetic drugs such as cocaine and amphetamines [62]. Vascular malformations such as aneurysms and arteriovenous malformations [AVMs] are much less common causes of hemorrhagic stroke in adults [67-68]. Risk factors for cerebrovascular disorders in children are quite different from adults. Congenital or acquired heart disease; congenital or acquired abnormalities of arterial structure such as arterial dissection, transient cerebral arteriopathy of childhood, moyamoya disease, and vasculitis; prothrombotic states; sickle cell anemia; and infection are common

risk factors for arterial ischemic stroke in children [69-71]. In neonates, maternal and fetal physiologic factors associated with pregnancy likely contribute to the risk of arterial ischemic stroke, as do congenital heart disease, prothrombotic states, maternal infection, and placental abnormalities [72]. Pediatric cerebral venous sinus thrombosis has been associated with dehydration, prothrombotic states, head and neck infection, trauma, surgery, malignancy, and inflammatory conditions [73]. Hemorrhagic stroke in children is commonly associated with vascular malformations such as AVMs, aneurysms, and cavernous malformations, although hematologic abnormalities and other medical conditions can be precipitants [74]. In contrast, the cause of hemorrhagic stroke in term neonates is often unknown [75].

TREATMENT OF CEREBROVASCULAR DISORDERS

The acute treatment of stroke depends on the mechanism of injury. In adults with arterial ischemic stroke, intravenous infusion of tissue plasminogen activator [tPA], a drug that lyses blood clots, is the only FDA approved treatment. Widespread use of this drug is limited by the need to administer it within 4.5 h of stroke symptom onset [76], and as a result fewer than 5% of adults with acute stroke receive this treatment [77]. Other acute stroke treatments include aspirin, intra-arterial administration of tPA [78], mechanical clot disruption, and endovascular removal of thrombus with a clot-retrieval device [79]. Surgical decompression can be beneficial in patients with space-occupying infarction [80]. Ongoing studies are evaluating the role of therapeutic hypothermia in the management of acute stroke [81].

To prevent future strokes, treatment with an antiplatelet or anticoagulant medication is recommended [78 Albers GW et al. 2008], in addition to treatment of stroke risk factors such as hypertension, elevated cholesterol, and diabetes. Stent placement may also be useful for secondary prevention in selected cases of arterial stenosis [79]. None of these treatments have been studied in children, although the use of antiplatelet or anticoagulant medications for secondary prevention is recommended in most cases [82]. Chronic transfusion therapy has been shown to prevent stroke in children with sickle cell anemia [83], and revascularization surgery for moyamoya disease also prevents stroke recurrence [84]. Anticoagulation is the treatment of choice for acute cerebral venous sinus thrombosis in adults and children [78].

Following intracerebral hemorrhage, acute treatment may include surgical evacuation of hemorrhage, placement of a temporary ventriculostomy catheter if obstructive hydrocephalus develops, and intraventricular infusion of thrombolytic medications to augment clearance of blood from the ventricles. Infusion of activated recombinant factor VII [a coagulant] has been shown to decrease the amount of hemorrhage expansion and garnered much recent enthusiasm, but this treatment has not consistently been associated with improved outcome [85]. Certain patient populations may benefit from this treatment, but identification of these groups will require further study. Preventative treatment of recurrent hemorrhage may include surgical clipping or endovascular coiling in the case of aneurysms and endovascular embolization, surgical resection, or treatment with stereotactic radiosurgery in the case of arteriovenous malformations. Supportive measures following any type of cerebrovascular insult include maintenance of cerebral perfusion pressure with intravenous fluids, avoidance of hypoglycemia or hyperglycemia, and avoidance of fever.

TREATMENT OF ISCHEMIC STROKE

Therapeutic as well as other strategies in the treatment and prevention of stroke has improved. The knowledge of role of inflammation and immune system in stroke and cerebrovascular disease has opened the door for research using *immunomodulatory* approaches in the treatment and prevention of stroke. There is only one Food and Drug Administration [FDA] approved treatment for ischemic stroke, the thrombolytic agent rt-PA that enhances brain reperfusion. Neuroprotective trials have been disappointing, with NXY-059—a free radical scavenger being the latest casualty [86-87; <http://www.astrazeneca.com>, 2007]. It is of note that a major limitation of hypothermia therapy for stroke, another form of neuroprotective therapy, has been the excess rate of pneumonic complications that has precluded its use, especially in the elderly. *Immune modulation* approaches would seem to be worthwhile pursuing based on the significance of the immune response on tissue and clinical outcomes after stroke. To date, therapeutic strategies for acute ischemic stroke have focused on preventing the recruitment and trafficking of neutrophils into ischemic lesions through inhibition of cellular adhesion molecules, however without success [88]. Enlimomab, a murine monoclonal antibody against ICAM-1, administered within 6 h of onset and continued for 5 days, led to worse stroke outcome: the antibody itself elicited a strong and adverse inflammatory reaction [89]. UK-279,276, an antibody that binds selectively to the CD11b/CD18 integrin on neutrophils was not effective in the Acute Stroke Therapy by Inhibition of Neutrophils [ASTIN] study [90]. It is possible that partial inhibition of neutrophils may be more effective than complete blockade.

Other *immune modulatory* approaches could theoretically include leukocyte-depleting strategies [not appropriate for the clinical population] and general strategies aimed at suppressing immune function [e.g., steroid treatment]: these have not proven to be effective in stroke and increase the risk of secondary infection. The antibiotic agent doxycycline appears to reduce leukocyte adhesion and was found to be beneficial in a temporary occlusion experimental stroke model, but has not so far been tested in the clinical setting [91].

Recombinant human interferon beta-1a [IFN-b1a] is an FDA approved treatment for patients with relapsing remitting multiple sclerosis. IFN-b1a inhibits pro-inflammatory cytokines and prevents BBB disruption and is currently being studied in ischemic stroke in a phase 1 safety study. This agent could lessen reperfusion injury after stroke [and possibly hemorrhagic transformation of infarcts which can be a complication of rt-PA therapy]. It too has been noted in a number of observational studies that patients who are taking a statin before a stroke have a better outcome than those who are not [92]. It has been proposed that, apart from cholesterol lowering, statins work through other mechanisms that include *immune modulation* [decreased inflammation], decreased oxidative stress, inhibition of the thrombogenic response and increased atherosclerotic plaque stability. In experimental stroke models statin administration has resulted in reduced size of brain infarcts [93].

TREATMENT OF HEMORRHAGIC STROKE

There are few proven treatments for intracerebral hemorrhage, the main management issue being strict control of hypertension. Surgical evacuation is usually reserved for

cerebellar hematomas, which show a mass effect [as clinically evidenced by a declining level of consciousness]. The coagulation factor Factor VIIa did show some initial promise in phase 2 studies [94] but was recently abandoned [<http://www.novonordisk.com>, 2007]. In terms of *immune modulation*, anti-inflammatory drugs such as COX-2 inhibitors, tacrolimus and fucoidan may reduce peri-hematoma inflammation and improve outcome in experimental stroke models [95]. Administration of the lipid-lowering drug atorvastatin has also been associated with improved outcome after experimental intracerebral hemorrhage, perhaps in part through modulation of inflammation in the peri-hematoma region [96]. For subarachnoid hemorrhage surgical clipping of the causative aneurysm or resection of the arteriovenous malformation is the mainstay of treatment. Endovascular coiling of the aneurysm can also be performed. Post-operative infection [either brain or respiratory] is an uncommon complication and not believed to be any more common than after other invasive surgical procedures. Hypervolemic-hemodilution and hypertensive [HHH] therapy is used to prevent spasm. There may be a role for anti-inflammatory measures for the prevention of vasospasm and delayed cerebral ischemia, as shown in a recent pilot study of patients treated with statins [97].

USE OF STEM CELLS IN TREATMENT CEREBROVASCULAR DISEASES

Stem cell therapy is an emerging therapeutic modality in the treatment of stroke. Its basis stems from the observation that certain parts of the adult brain are capable of regeneration [a relatively recent discovery]. Neurogenesis in the adult brain has been demonstrated in the dentate nucleus of the hippocampus and the subventricular zone. In a study of patients with ischemic stroke, neurogenesis was demonstrated in the ischemic penumbra, where cells were found to preferentially localize to the vicinity of blood vessels. These findings are suggestive of poststroke compensatory neurogenesis, which may contribute to recovery after the insult. While the regenerative capacity of certain parts of the brain has been demonstrated, it is clear that this endogenous repair process is unable to overcome the devastating damage to brain tissue that occurs after acute, severe stroke. Cell-based therapies have the potential to open up new avenues of treatment in this arena. Targets for stem cell therapy include neuroprotective approaches aimed at protecting at-risk tissue during the acute phase of stroke, as well as neuroreparative approaches which may involve direct replacement of damaged brain tissue, or alternatively promotion of the brain's endogenous repair processes. [98]

Stem cell-based therapeutics for stroke have recently commenced in the clinic [99]. Despite the advance in our scientific knowledge, cerebrovascular diseases are a major cause of death and disability worldwide. Accordingly, finding a novel treatment, which can be effective well beyond the acute 3-h window after cerebral attack, is being heralded as a unique treatment regimen in the clinic. In recent years, the advancement of stem cell therapy from the laboratory to the clinic has been guided by research recommendations from Stem Cell Therapeutics as an Emerging Paradigm for Stroke (STEPS) [100]. The consortium guidelines are designed to enhance the safety and efficacy of stem cellbased therapeutics as we translate these novel treatments to stroke patients. The endothelial progenitor cells can take advantage of STEPS as the cells move toward clinical application. Some evidence-based interventions during the acute phase of stroke such as organised stroke unit care and

reperfusion therapies with intravenous recombinant tissue plasminogen activator have been recognized to improve the outcome including survival and residual disability that are available only for a minority of patients [101].

Moreover, once cell damage from stroke is established, little can be done to restore pre-stroke conditions. In last years, in the background of this imperative clinical need, hundreds of studies have been published investigating therapeutic potential of stem cell transplantation, on the basis of animal studies showing that cells transplanted to the brain not only survive but also lead to functional improvement in different neurodegenerative diseases models [102].

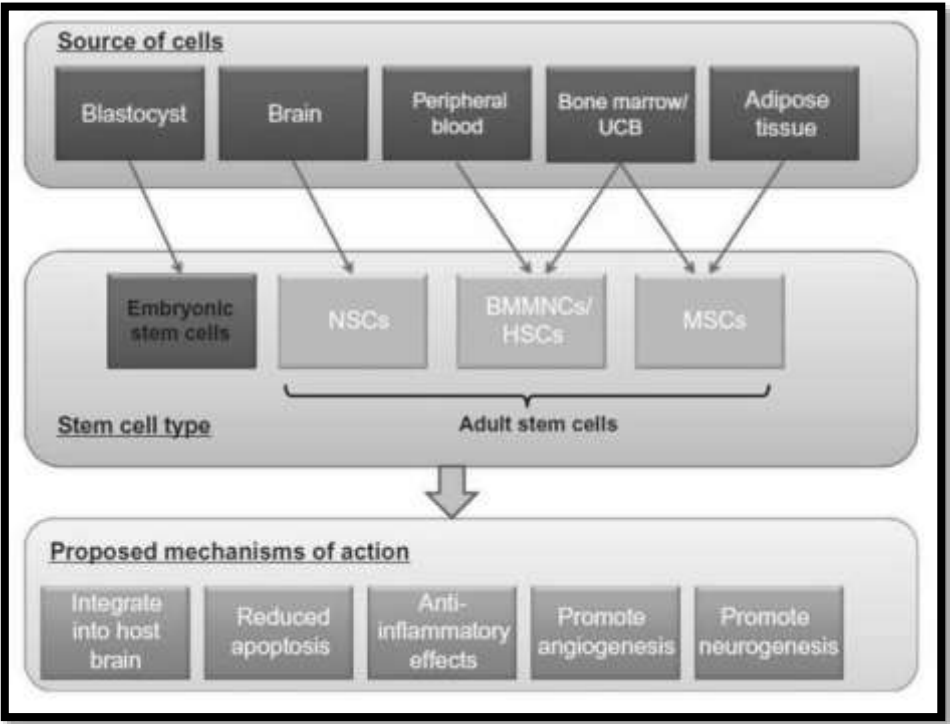
Transplanted cells have been hypothesised to be effective not only by cell replacement within the damaged host tissue but also by providing trophic and neuroprotective support as well as immunomodulatory mediators [103]. The existence of endogenous neurogenesis in an adult vertebrate brain was described by Luskin et al. and Alvarez-Buylla et al., who demonstrated for the first time the presence of neuronal stem cells [NSCs] in the adult rodent subventricular zone [SVZ] that migrate out to the olfactory bulb and integrate into the existing neuronal network [rostral migratory stream, RMS] [104]. Recently, similar cell populations were identified in the adult human SVZ, with evidence of neurogenesis in the mammalian SVZ and subgranular zone of the dentate gyrus although the existence of the RMS still remains controversial in the human brain [105]. Recent studies have detected NSCs in other brain regions, such as the striatum, spinal cord and neocortex [106]. Interestingly, the stroke-damaged adult rodent brain preserves some replacement capacity mediated by endogenous NSCs. It is reported that stroke increases the number of newly generated cells in the SVZ in animals [107] and NSCs are able to generate new striatal neurons that migrate to the site of damage for several months after stroke [102].

Stroke infarct triggers early expansion of the progenitor pool increasing the fraction of proliferating SVZ cells and shortening the cell cycle length [108]. In addition to NPCs, stroke induces adult ependymal cells to proliferate and acquire features of radial glial cells [109]. Strikingly, stroke-induced neurogenesis has recently been observed in the adult human brain, even among the elderly [110]. Different neurotrophic factors [111] are responsible for endogenous neurogenesis stimulation after stroke. Moreover, neuroblasts migrate to the tissue adjacent to the infarct [112] attracted by matrix metalloproteinases [MMPs], particularly MMP2 [113], produced by the compromised endothelial cells and by neuroblasts themselves in a loop of endothelial–neuronal interaction. The neuroblasts protective role is reinforced by the fact that they express doublecortin, a marker of cell migration that is shown to be neuroprotective [113]. In addition, chemotactic signals, particularly SDF/CXCR4, are known to contribute to cell migration. There is also evidence of the beneficial impact of exercise on the functional plasticity after stroke, by providing neurotrophic support to the lesion environment and promoting neural repair. Exercise-induced neurogenesis was confirmed in humans by measuring exercise-specific changes in cerebral blood volume in the adult human dentate gyrus [114]. The majority of studies to date have shown relatively limited cell replacement from endogenous NSCs and mobilising endogenous NSCs is relatively a new approach.

In contrast, researchers has been in progress for decades to replace lost neural cells by transplantation of stem cells from different sources such as foetal embryonic stem cells, neuroepithelial or teratocarcinoma cell lines by using different methods for their isolation and culture. Potential advantages to this approach may include greater control over cell fate, the ability to deliver any desired number of cells and reduced risks associated with mitogen

infusion. The major goal is reconstitution of the complex and widespread neuronal–glial–endothelial interrelationship may require access to a broader array of cell lineages, since stroke affects multiple cell types including neurons, glia and endothelial cells. Thus, ideally, cells should need to maintain initially an immature state and differentiate into several specific cell types after engraftment. The host environment plays an important role in this issue by generating appropriate neurogenic signals. Lack of these may divert the cell fate predominantly toward the glial phenotype. Moreover, stem cell transplantation could enhance clinically valuable improvements through several other mechanisms, including growth factor production, endogenous damage-induced plasticity, and local re-innervation, partially due to neuronal replacement [Figure 1.4].

However, the potential success of transplantation in stroke, in comparison to neurodegenerative diseases, is influenced by some critical issues including anatomy, localization and size of infarct area, time of transplantation, vascular supplies, route and site of implantation and patient selection. Despite stroke injury being focal, the neuronal degeneration in stroke is not selective but involves different neuronal populations, including glial and endothelial cells, and disrupts various anatomical pathways, including both white and grey matter that need to be restored. In contrast, most experimental studies are conducted using a middle cerebral artery stroke model that presents mostly the striatum and, in a minor part, cortex damage, and only a few authors investigated cell therapy for cortex infarcts [115].



Abbreviations: UCB, umbilical cord blood; NSC, neuronal stem cell; BMMNC, bone marrow mononuclear cell; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell. [Banerjee et al. 2012]

Figure 4. Stem cell types used in stroke trials and the proposed mechanisms of action.

Table 1.3. Summary of ongoing or completed [but unreported] acute/subacute stroke trials

Cell type	Study design	Expected no of patients	Timing of delivery poststroke	Route of delivery	Clinical trial identifier	Trial status
Acute and subacute trials (4 weeks postevent)						
BMMNC	PII, R-OL	120	7–30 days	IV	NCT01501773	Complete
MSCs	PII, NR-OL	120	7–14 days [infarct] or 10–21 days [ICH]	IV, then intrathecal 7 days later	NCT01389453	Recruiting
CD34+	PI/II, NR-OL	10	7 days	IA	NCT00535197	Recruiting
MSCs	PI/II, R-DB	78	,10 days	IV	NCT01091701	Not yet recruiting
MultiStem	PII, R-DB	140	1–2 days	IV	NCT01436487	Recruiting
ALD-401	PI/II, R-DB	100	13–19 days	IA	NCT01273337	Recruiting

Note: Multi-Stem and ALD-401 are commercially developed stem cell lines.

Abbreviations: BMMNC, bone marrow mononuclear cells; MSCs, mesenchymal stem cells; OECs, olfactory ensheathing cells; EPCs, endothelial progenitor cells; NR, nonrandomized; OL, open-label; PI and II, phase I and II trials; R, randomized; SB, single-blind; DB, double-blind; IV, intravenous; IA, intra-arterial; IC, intracerebral; ICH, intracerebral hemorrhage.

Thus, there are not any conclusive results on the possibility of restoring cortical damage and thereby memory and behavioural functions. It can be argued that infarcts associated with cortical involvement are larger and require the reestablishment of essential connections. The optimal time for cell engraftment after stroke is not yet well defined because of the dynamic modifications of the ischemic lesion's environment over time [116]. Excitotoxic neurotransmitters, free radicals as well as proinflammatory agents are released in acute phase [117]. The activated inflammatory response, leading to microglial reaction, together with apoptosis limits both the growth and survival of transplanted cells and endogenous neurogenesis [118]. Otherwise, the increased release during the stroke acute phase of cytokines and neurotrophic factors such as granulocyte colony stimulating factor [119] potentially could favour cell implant survival and growth. In experimental stroke, it has been observed that during the first 2–3 weeks and even longer, the peri-infarct cortex upregulates gene expression related to the modulation of neuronal growth, involving increased expression of cytoskeletal proteins, angiogenesis, cell proliferation, differentiation and migration from SVZ [120]. On the other hand, transplantation subsequent to the acute phase encounters difficulties due to the hostile lesion environment generated from the scar tissue formation. Stroke spontaneous recovery depends on brain's plasticity in terms of replacement of afferent and efferent connections and synaptogenesis, which occur early after stroke and last for months or years. There is evidence that these endogenous repair mechanisms can be enhanced by transplantation. In conclusion, it is rational to delay transplantation until neurological deficits reach a plateau and any further spontaneous recovery is unlikely. In studies seeking to demonstrate the generation of new graft-derived circuitry, even greater delays may be employed after injury to ensure stable long-term behavioural deficits prior to transplantation [120].

In conclusion, the field of cell therapy is a very exciting area to develop potential new therapies for stroke. However, the field is still too much in an embryonic and starting stage to make conclusions on its efficiency as a treatment for stroke. There are many unanswered questions surrounding the best cell types, optimal delivery routes, therapeutic time windows and appropriate patients.

STEM CELL THERAPIES FOR CEREBROVASCULAR DISEASES UNDER CLINICAL TRAILS

Routine use of stem cells at the bedside for stroke patients is an exciting prospect, though realistically remains a long way off. The future may well see the availability of “off-the shelf” stem cell products available for immediate use. Perhaps patients will receive individually tailored stem cell products, engineered to secrete specific trophic factors, to suit their specific subtype and duration of stroke. There is clearly a long way to go in both preclinical and clinical research, and long-term biosafety measures will be an essential consideration in this regard.

A number of clinical trials have now begun to demonstrate the safety and feasibility of different approaches to stem cell therapy. However, there are a large number of unanswered questions that remain. The great variation in the stem cell trials completed to date means that it is difficult to make any meaningful comparisons between them. Future clinical trials will need to start trying to address the important outstanding questions. It is imperative that trials are therefore designed with this in mind. Careful planning and collaborative work need to take place in order to maximize the likelihood of getting a useful answer from these studies. This includes such considerations as incorporation of a dose-escalation design and comparisons between different methods of delivery as well as different time points for delivery. The Stroke Therapy Academic Industry Roundtable [STAIR] II meeting elucidated the important considerations when designing future phase IIb studies. Many of these recommendations are particularly relevant to the field of stem cell research. They emphasized that “lack of establishing the optimal dose, duration of therapy, and time window may have contributed to the failure of neuroprotection trials.” Therefore, refinement and identification of the target population is essential. In addition, narrowing selection criteria in phase IIb studies to target patients more likely to respond based on clinical and imaging characteristics may optimize the chances of detecting a biologically relevant drug effect [STAIR IV recommendations]. Subsequent to these public cations, the Stem Cell Therapies as an Emerging Paradigm in Stroke [STEPS] meeting sought to develop a framework specifically to help guide design of future preclinical and clinical studies in the field of cell-based therapies. These recommendations from experts from the preclinical and clinical arenas should be carefully borne in mind before commencing any future trials.

In conclusion, there have been significant advances made in the field of stem cell research over the last two decades, with evidence of significant benefits in both acute and chronic animal models of stroke. Translation to clinical practice, however, remains a long way off. A number of trials are under way, though future work will need to concentrate on overcoming the still-significant challenges standing in the way of this becoming a realistic treatment option for the future.

ADVANTAGES OF STEM CELL THERAPY TO PATIENTS

A crucial question for upcoming clinical trials is which patients will benefit from stem cell therapy. The pathological type and anatomical location of the stroke are important issues, which are discussed in more detail below. In addition, demographic differences, in particular differences in age and gender, are also likely to affect how patients respond to treatment. The vast majority of preclinical work has been conducted on young, previously healthy, male animals. Elderly patients, who represent the largest subset of stroke victims, may not derive the same benefit as that seen in such animal studies. As stated previously, the majority of preclinical work has focused on ischemic rather than hemorrhagic strokes. In view of this, the ongoing and completed clinical trials have almost exclusively concentrated on ischemic stroke patients. Of the published clinical trials to date, only one has included any patients with hemorrhagic strokes [121].

Within the ischemic stroke subgroup, the majority of preclinical evidence for benefit has been obtained from animal models with predominantly striatal infarcts. A smaller number of trials have investigated cortical models of stroke, with mixed results [122]. Cortical strokes are associated with larger, more damaging infarcts, and it may be that these are too large to affect any repair. A direct comparison between subcortical and cortical infarcts in addition to various doses of cells would be useful in order to optimize patient selection. For example, one preclinical study that compared anatomical location found the benefit of stem cell therapy to be more pronounced in striatal infarcts, rather than larger cortical lesions [123]. Further issues to consider when designing clinical trials are stability of patients and associated comorbidities. Patients with larger strokes are commonly medically unwell due to multiple reasons [eg, aspiration pneumonia, cardiac arrhythmias], which could preclude them from entry into acute trials despite being eligible. Furthermore, multiple comorbidities are also likely to affect the response of the patient to stem cell therapy.

SUMMARY

Stroke is the leading cause of disability worldwide, the second most common cause of dementia and the third leading cause of death. It has enormous clinical, social, and economic implications and demands a significant effort from both basic scientists and clinicians in the quest for understanding the underlying pathogenic mechanisms, and thereby adopting suitable preventive measures and successful therapies. Once considered exclusively a disorder of blood vessels, growing evidence has led to the realization that the biological processes underlying stroke are driven by the interaction of neurons, glia, vascular cells, and matrix components, which actively participate in mechanisms of tissue injury and repair. Stroke is a serious neurological disease, and constitutes a major cause of death and disability throughout the world. The pathophysiology of stroke is complex, and involves excitotoxicity mechanisms, inflammatory pathways, oxidative damage, ionic imbalances, apoptosis, angiogenesis and neuroprotection. The ultimate result of ischemic cascade initiated by acute stroke is neuronal death along with an irreversible loss of neuronal function. Therapeutic strategies in stroke have been developed with two main aims: restoration of cerebral flow and the minimization of the deleterious effects of ischemia on neurons. Intense research spanning

over the last two decades has witnessed significant therapeutic advances in the form of carotid endarterectomy, thrombolytics, anticoagulant therapy, antiplatelet agents, neuroprotective agents, and treating associated risk factors such as hypertension and hyperlipidemia. However, the search for an effective neuroprotectant remains frustrating, and the current therapeutic protocols remain suboptimal. Till date only one FDA-approved drug is available for ischemic stroke; i.e., the serine protease tissue-type plasminogen activator [TPA], the utility of which is limited by the short therapeutic window. As new targets are identified, new opportunities emerge that build on an appreciation of acute cellular events acting in a broader context of ongoing destructive, protective, and reparative processes.

At the onset of the 21st century, it is the third-leading cause of death in most developed countries and the primary cardiovascular cause of death in Japan and China. The health burden of the disease is staggering as loss of a productive life inflicts a heavy toll on patients, families, and society. Yet this disease has no effective therapy beyond a limited group of patients [5%] who are treated with thrombolytics, which have significant adverse effects. This situation prevails despite intense research efforts and numerous clinical trials that have attempted to develop drugs to reduce morbidity and mortality from stroke. So far, drug development efforts have targeted modulators of ion channels [Ca^{2+} and Na^{+}], scavengers of oxygen radicals, and antagonists of excitotoxic neurotransmitters [primarily glutamate and glycine receptors].

However, current therapeutic strategies for stroke have been largely unsuccessful. One possible explanation is that research and pharmacological management have focused on very early events in brain ischemia. However, clinical trials with modulators of these targets have failed so far because of lack of efficacy, adverse effects, or other developmental difficulties. Debate on the reasons for this grim reality has sprung up in recent meetings, with finger-pointing about major possible causes of failure, including incorrect animal models, misidentified mechanisms of action, poor clinical designs, inadequate timing of treatment, and other variables. Moreover the brain ischemia and trauma elicit strong inflammatory reactions driven by both external and brain cells. The recognition of inflammation as a fundamental response to brain ischemia provides novel opportunities for new anti-inflammatory therapies. Two important pathophysiological mechanisms involved during ischemic stroke are oxidative stress and inflammation. Brain tissue is not well equipped with antioxidant defenses, so reactive oxygen species and other free radicals/oxidants, released by inflammatory cells, threaten the tissue viability in the vicinity of the ischemic core. With increase in our understanding regarding the roles infections and immune reactions play in the brain milieu this may also have an impact on the treatment of neuroinjuries and ancillary brain diseases.

Acute ischemic stroke is treatable, and our ability to treat patients with ischemic stroke continues to improve. Perhaps the most important has been the widening of the time window for both intravenous thrombolysis as well as endovascular arterial recanalization treatments. This change in the expansion of the time window has major implications because it could dramatically increase the number of potential patients for treatment. Further expansion of the time window is possible with the likelihood that imaging will provide the necessary information for identifying suitable, individual patients. The basic imaging approaches to acute ischemic stroke patients have evolved slightly since the first edition. More importantly, there has been a deepening of our understanding of the significance of the findings observed on standard and advanced diagnostic techniques like CT and MRI. Here we will focus on the

ischemic penumbra, and the other on how to use imaging to help guide endovascular therapy. All of this will reflect improved understanding of what imaging can provide the physician caring for the stroke patient to help make the wisest decision with respect to interventions that are being considered. The field continues to evolve, and better outcomes are possible and even likely. Indeed the burgeoning opportunities for treatment are so substantial that a major issue is whether there are enough trained physicians to provide optimal care to patients with acute ischemic stroke, especially those with the most severe strokes.

Cerebrovascular diseases are the leading cause of mortality and morbidity worldwide. These diseases pose many clinical challenges and even experienced clinicians can arrive at the point where work-up, treatment, or prognostic thinking falters. In the chapters coming ahead we will highlight the latest trends in preventive and treatment measures regarding patients prone to or suffering from stroke, embolism, thrombosis, hemorrhage, and other critical cerebrovascular ailments. While progress has been made in prevention and supportive care, efforts to protect the brain from cell death have not succeeded completely hence, no new treatment has made it from bench to bedside since tissue plasminogen activator was introduced some 18 years ago. Thus there is urgent challenge before the scientific fraternity to come up with new ideas and ways to treat stroke and protect brain from cell death. Most therapeutic approaches developed in the laboratory have focused on protecting neurons from the main pathogenic mechanisms causing ischemic injury, such as excitotoxicity, oxidative stress, inflammation or apoptosis. These experimental treatments have shown some progress in large clinical trials, an outcome that has sparked a lively debate about the promise of neuroprotection in stroke therapy. Unlike traditional therapeutic approaches based on counteracting selected pathways of the ischemic cascade, endogenous neuroprotection relies on coordinated neurovascular programs that support cerebral perfusion, mitigate the harmful effects of cerebral ischemia and promote tissue restoration. Understanding how the brain triggers and implements these protective measures may advance our quest to treat stroke and open a new era in stroke therapeutics. Here in this book we will describe different modalities by which the brain protects itself, aiming to provide a synthesis of the different mechanisms and highlighting their potential relevance for the future of stroke therapy. Leading-edge scientific research from across the globe and the possible mechanisms involved in treatment of stroke will be presented in this book to the audience. Finally we will focus on the role of different medicines and the natural herbs in the prevention and treatment of Cerebrovascular Diseases with focus on stroke.

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Chapter 17

IMMUNOBIOLOGY OF STROKE

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ABSTRACT

The immune-privileged status of the CNS has evolved to maintain homeostasis required for neural function and host defense. The inability to generate robust and potentially harmful adaptive immune responses therefore requires a primary reliance for host defense on the sequestered and moderate innate responses of microglia, astrocytes, and other resident innate cells. Immunity and inflammation are key elements of the pathobiology of stroke, while the immune system participates in the brain damage produced by ischemia, the damaged brain, in turn, exerts a powerful immunosuppressive effect that promotes fatal inter-current infections and threatens the survival of stroke patients. Inflammatory signaling is instrumental in all stages of the ischemic cascade, from the early damaging events triggered by arterial occlusion, to the late regenerative processes underlying post-ischemic tissue repair. Recent developments have revealed that stroke, like multiple sclerosis, engages both innate and adaptive immunity. But, unlike multiple sclerosis, adaptive immunity triggered by newly exposed brain antigens does not have an impact on the acute phase of the damage. Nevertheless, modulation of adaptive immunity exerts a remarkable protective effect on the ischemic brain and offers the prospect of new stroke therapies. However, immunomodulation is not devoid of deleterious side effects, and gaining a better understanding of the reciprocal interaction between the immune system and the ischemic brain is essential to harness the full therapeutic potential of the immunology of stroke. Our understanding of the interactions between resident and peripheral immune cells, neurons, and glial cells and their implications for host defense, tissue repair, and neurodegeneration is still in its infancy. Increasing evidence shows that the central nervous system and the immune system interact in complex ways, and better insight into these interactions may be relevant to the treatment of patients with stroke and other forms of central nervous system injury. In

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addition, the immune system actively participates in the acute pathogenesis of stroke. Thrombosis and hypoxia trigger an intravascular inflammatory cascade, which is further augmented by the innate immune response to cellular damage occurring in the parenchyma. The activation of innate immunity after stroke sets the stage for an adaptive immune response directed against brain antigens. The pathogenic significance of adaptive immunity and its long-term effects on the postischemic brain remains unclear. Further research will be required to determine what role, if any, immunity has in long-term outcomes after stroke, but elucidation of potential mechanisms may open promising avenues for the development of new therapeutics to improve neurological recovery after brain injury. Recent clinical and experimental studies have highlighted a complex role for the immune system in the pathophysiological changes that occur after acute stroke. Sensors of the innate immune system such as Toll-like receptors, or effectors such as the lectin pathway of complement activation and innate immune cells, are activated by brain ischaemia and tissue damage, leading to amplification of the inflammatory cascade. Activation of the adaptive arm of the immune system, mediated by lymphocyte populations including T and B cells, regulatory T cells, and $\gamma\delta$ T cells, in response to stroke can lead to deleterious antigen-specific autoreactive responses but can also have cytoprotective effects. Acute stroke is followed by a complex interaction between the brain and the immune system. Damage-associated molecular patterns are released after neuronal damage, and activate the innate and adaptive arms of the immune system. Different populations of lymphocytes can exert beneficial or detrimental functions after acute stroke, although the underlying mechanisms are not fully elucidated. Stroke can lead to immunodepression, increasing the risk of infections such as pneumonia. The increased incidence of infections is observed after acute stroke, and might result from activation of long-distance feedback loops between the CNS and peripheral immune organs, which are thought to play a part in stroke-induced immunodepression. Ongoing clinical trials are investigating whether the preventive use of antibiotics improves functional outcome after stroke. However, the delineation of the molecular interactions between the immune and nervous systems is proceeding rapidly and will yield translational application in the years to come.

BRAIN IMMUNE INTERACTIONS

To understand the fine-tuned bi-directional communication between the brain and the immune system is still a challenge for the Modern science. Brain-immune interactions take place in different organs, involving a wide range of cells and mediators, coordinated through sensory and effector pathways in the central nervous system [1-3]. The interactions work in both directions to maintain a healthy state of body and brain in the face of diverse, harmful challenges from injury, allergens, infective agents, foodstuffs and toxins. Dysfunction and inappropriate regulation of inflammatory or neuronal responses underlie many diseases that have become more prevalent in recent decades. Recent research has established a significant role for the immune system in several brain diseases including stroke, multiple sclerosis, tumors, mental disorders, Alzheimer's, and Parkinson's disease. In turn, mood disorders, stress, autonomic dysfunction, acute, and chronic brain injury have been linked with the development of organ failure, cancer, heart disease, systemic inflammatory conditions, infections, and hematological diseases further implicating dependent interrelationships between the immune system and the brain [4-8]. Both preclinical and clinical research has contributed significantly to our knowledge about these interactions, yet another major challenge is to translate multiple research findings into clinical benefit.

Murakami and colleagues present their research findings and their “gateway” theory of how regional neuronal responses can drive the migration of autoreactive T cells across the cerebrovascular endothelium to particular sites of the brain where they contribute to the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis [9]. They also show that regional neural stimulation can therapeutically prevent the gating through blood vessels. Anrather and colleagues have described how reprogramming of local and systemic immune mechanisms contributes to the induction of cerebral ischemic tolerance, a process that is characterized by protection against the ischemic injury after application of ischemic stress to one tissue or organ [10]. Appropriate reprogramming of key immune mechanisms could be used to develop novel stroke therapies including possible prevention of injury through stroke in vulnerable individuals. The research paper by Denes et al., demonstrates that brain injury, anesthesia, and surgical interventions have diverse systemic consequences, including altered leukocyte responses in several organs of the body and rapid mobilization of granulocytes [11]. This could have important implications for animal models of cerebral ischemia as well as for patients with brain injury or for those undergoing surgeries or exposed to prolonged anesthesia. Assas and colleagues discuss important aspects of neuro-immune communication and show how sensory fibers containing the neuropeptide calcitonin gene-related peptide (CGRP) shape the responses of macrophages, mast cells and other immune cells throughout the body and how these interactions contribute to immune defense and diverse inflammatory conditions [12]. This neuropeptide and the c class nerve fibers that contain it thus form a key pathway for bi-directional neuroimmune interactions and could form a target for future neuroimmune based therapies. Neuro-immune processes are likely to contribute to diverse pathologies in both the periphery and the brain leading to complex human diseases that affect millions of people worldwide. Understanding mechanisms of neuro-immune interactions could help to find appropriate therapies to some of these conditions.

BRAIN-IMMUNE INTERACTIONS AND ISCHEMIC STROKE

Mounting evidence indicates that the immune system has a key role in brain injury. A better understanding of the interactions between the immune system and the brain can aid physicians who care for patients with stroke and other forms of central nervous system (CNS) injury. In addition, advancing our understanding of the immunology of stroke promises to generate novel clinical strategies, as well as diagnostic and therapeutic approaches. Here in this chapter we will discuss the selected aspects of the interactions between CNS injury and immunity, focusing on its implications for novel therapeutic agents to modify the immune response to stroke. In addition, we high-light the many gaps in our understanding of the role of the immune system in CNS injury and examine promising avenues of future investigation.

Immune system plays an elementary role in the pathophysiological progress of ischemic stroke. It consists of innate and adaptive immune system. Activated within minutes after ischemic onset, innate immunity is responsible for the elimination of necrotic cells and tissue repair, while it is critically involved in the initiation and amplification of post-stroke inflammation that amplifies ischemic damage to the brain tissue. Innate immune response requires days to be fully developed, providing a considerable time window for therapeutic

intervention, suggesting prospect of novel immune-modulatory therapies against post-stroke inflammation-induced brain injury. However, obstacles still exist and a comprehensive understanding of ischemic stroke and innate immune reaction is essential. In this chapter, we will try to highlight the current experimental and clinical data depicting the innate immune response following ischemic stroke, mainly focusing on the recognition of damage-associated molecular patterns, activation and recruitment of innate immune cells, and involvement of various cytokines. In addition, clinical trials targeting innate immunity were also documented regardless of the outcome, stressing the requirements for further investigation.

IMMUNE ACTIVATION AND THE RISK OF STROKE

Several lines of evidence suggest that activation of the immune system may increase the risk of stroke (Table 1). Numerous prospective population-based investigations demonstrated a correlation between levels of inflammatory biomarkers (such as white blood cell count, fibrinogen, D-dimer, and C-reactive protein) and the risk of incident and recurrent stroke [13]. Biomarkers may allow identification of patient subgroups who derive greater or lesser degrees of benefit from standard medications, such as antiplatelet or lipid-modifying agents. In addition, improved knowledge about the link between inflammation and stroke may lead to better and more timely recognition of specially vulnerable populations, such as patients with recent infection or surgery who face a transiently heightened risk of stroke.

Furthermore, animal models demonstrate that atherosclerosis has an inflammatory component, and inhibition of the immune response to lipoproteins seems to reduce the progression of atherosclerosis. These observations suggest that inflammation may have a causal role in vascular injury and subsequent stroke, which would open the door for immune-modulatory agents as new tools to prevent stroke in these patients. However, observational data are notoriously prone to confounding, and animal models often do not apply well to humans. Clearly, a more detailed understanding of the complex relationship between inflammation and stroke is required to better assess the feasibility of immunomodulation as a potential tool for stroke prevention.

Inflammation is increasingly recognized as a possible pathway in the pathogenesis of atrial fibrillation, which is a leading cause of stroke [14]. Levels of C-reactive protein are elevated in patients with atrial fibrillation and are associated with incident atrial fibrillation and with its recurrence after ablation or cardioversion. Inflammatory pathways may promote atrial fibrillation by interacting with cell signaling cascades, causing ion channel dysfunction, impairing myocyte gap junctions, promoting atrial fibrosis, and recruiting leukocytes to cardiac tissue. The relationship between inflammation and atrial fibrillation is most likely bidirectional, with atrial fibrillation causing some degree of immune activation and inflammation. The prothrombotic state seen in atrial fibrillation may reflect this inflammation, and anticoagulation with heparinoids seems to reduce biomarkers of inflammation in patients with atrial fibrillation. On the other hand, perioperative treatment with glucocorticoids reduces the incidence of atrial fibrillation after cardiac surgery, which suggests that inflammation may also have a causal role in the pathogenesis of atrial fibrillation. Once patients develop atrial fibrillation, their risk of stroke varies in proportion to known clinical risk factors, such as congestive heart failure, hypertension, age, diabetes

mellitus, prior stroke, and peripheral vascular disease. However, levels of the proinflammatory cytokine interleukin 6 are also associated with stroke risk, suggesting that inflammation is an additional biomarker of stroke risk within this population. Given these data, physicians should be mindful that periods of heightened inflammation (such as acute medical illness or recent surgery) place patients at higher risk of atrial fibrillation and stroke. With further development, biomarkers of inflammation may help to stratify patients' risk of developing atrial fibrillation and stroke, allowing targeted screening, risk factor modification, and timely treatment. A better understanding of the interactions among atrial fibrillation, inflammation, and thromboembolism may lead to the development of therapeutic agents that modulate inflammatory pathways to reduce the risk of atrial fibrillation and stroke.

IMMUNE SIGNALING DURING ACUTE INFARCTION

Besides its background role in stroke risk, the immune system actively participates in the acute pathogenesis of stroke [15]. Independent of any immune response, brain ischemia quickly causes failure of ion pumps, overaccumulation of intracellular sodium and calcium, loss of membrane integrity, and necrotic cell death. In addition, arterial occlusion immediately leads to intravascular hypoxia, changes in shear stress, and the production of reactive oxygen species, all of which in turn activate the coagulation cascade, complement, platelets, and endothelial cells. This results in a vicious cycle, with fibrin formation entrapping platelets and leukocytes and causing further vascular occlusion. In addition, oxidative stress reduces the bioavailability of nitric oxide, undermining its protective role in promoting vasodilation and inhibiting platelet aggregation and leukocyte adhesion, causing further vascular occlusion and ischemia. Central in this cascade of events is the translocation of P-selectin, an adhesion molecule whose expression on the surface of platelets and endothelial cells rapidly leads to cell adhesion. Trafficking of inflammatory cells into the perivascular space is facilitated by down-regulation of junctional proteins that maintain the integrity of the endothelial lining and the blood-brain barrier. Involvement of the perivascular space then activates resident macrophages and mast cells, leading to the release of vasoactive mediators and proinflammatory cytokines, which in turn recruit and promote the infiltration of more leukocytes. As cells die of ischemia, they release signals that further activate the immune system.

Extracellular accumulation of adenosine triphosphate released from dying cells activates microglia, which develop characteristics of macrophages and release proinflammatory mediators. Numerous normally intracellular components serve as danger-associated molecular pattern molecules on their release from dying cells, and these molecules activate toll-like receptors and scavenger receptors on microglia, perivascular macrophages, dendritic and endothelial cells, and infiltrating leukocytes. This activation induces the expression of proinflammatory molecules and primes dendritic cells for antigen presentation. Such proinflammatory changes are initially counterbalanced by the release of neurotransmitters, which activate anti-inflammatory receptors on microglia, and by the presence of cell-cell interactions between microglia and adjacent neurons, which usually keep microglia quiescent. However, as ischemic cell death progresses, neurons die and neurotransmitters are depleted, releasing this brake on proinflammatory signaling.

The clinical implications of the immediate immune involvement in the ischemic cascade are unclear. On the face of it, proinflammatory signals seem to promote microvascular occlusion and should tend to increase the size of the resulting infarct. In fact, in experimental models of stroke, mice deficient in adhesion receptors or complement subunits seem to be protected from acute ischemia, and healthy mice treated with inhibitors of adhesion molecules or the complement cascade also develop less ischemic brain injury. In addition, mice engineered to lack selected T-cell subgroups are protected from ischemic damage to the penumbral zone around areas of infarction [16]. Available data indicate that the protective effect of lymphocyte suppression does not stem from an inability to propagate thrombus and that no significant differences in cerebral blood flow exist between healthy and lymphocyte-deficient mice [17, 50]. It is possible that lymphocytes instead produce cell damage directly or through proinflammatory signaling and activation of downstream microglia and macrophages. Or, the early damage associated with lymphocyte infiltration of the ischemic brain may be due to the natural killer T-cell subtype that harbors a simplified T-cell receptor and may not require antigen processing. The available data do not provide a clear picture of how lymphocytes participate in acute infarction.

Clinical attempts to explicitly modify the immune response after stroke (such as trials of recombinant neutrophil inhibitory factor or antibodies against adhesion molecules) have been ineffective to date, and these failures highlight the complexity and redundancy of the pathways involved in the immune response to stroke. On the other hand, observational data and a randomized clinical trial indicate that acute use of statin medications at the time of stroke improves long-term outcomes and reduces mortality. Because this time window is not consistent with the lipid-lowering effects of statin medications, the benefit of their use during the acute stage of stroke has been attributed to their anti-inflammatory properties. This suggests that, despite the absence of specific clinical strategies or drugs proven to beneficially modulate immune functioning during acute brain infarction, further elucidation of this complex interplay may yield more sophisticated and pleiotropic therapeutics to augment the limited repertoire of antithrombotic agents available to physicians today.

Inflammatory Signaling in the Early Post-Ischemic Period

Post-ischemic inflammation is characterized by an orderly sequence of events involving the brain, its vessels, the circulating blood, and lymphoid organs. Inflammation is an integral part of the cascade of events triggered by ischemia and reperfusion (I/R) (Box 1). The inflammatory process begins in the intravascular compartment immediately after arterial occlusion, when the ensuing hypoxia, changes in shear stress, and production of reactive oxygen species (ROS) trigger the coagulation cascade, and lead to activation of complement, platelet and endothelial cells (EC) [18-21]. Intravascular formation of fibrin, traps platelets and leukocytes leading to microvascular occlusions and within minutes after ischemia, the adhesion molecule P-selectin is translocated to the surface membrane of platelet and EC12, and proinflammatory signals are rapidly generated (Table 1). Oxidative stress in EC reduces the bioavailability of nitric oxide (NO), a potent vasodilator and an inhibitor of platelet aggregation and leukocyte adhesion [22]. Loss of the beneficial effects of NO exacerbates intravascular plugging and aggravates the ischemic insult by reducing blood flow to the ischemic territory [23-24]. Furthermore, oxidative stress leads to constriction of pericytes,

contractile cells that replace myocytes in capillaries, producing microvascular occlusions [25]. Oxidative stress and inflammatory mediators also alter the permeability of the BBB. Pinocytotic vesicles increase in the cytoplasm of EC, enhancing trans-endothelial transport [26].

Proteases are expressed in vascular cells and released by leukocytes, whereas junctional proteins that seal adjacent EC are downregulated, facilitating extravasation of proteins and cells through the paracellular route (Figure 1) [26]. In the perivascular space, I/R activates perivascular macrophages and mast cells. Mast cell degranulation releases vasoactive mediators (histamine), proteases, and TNF, while activated macrophages release proinflammatory cytokines [27-29] (Box 2) (Figure 1). These proinflammatory mediators contribute to the endothelial expression of adhesion molecules and to the BBB damage that promotes the infiltration of leukocytes (neutrophils, lymphocytes and monocytes) [30].

Ischemic Cell Death Activates Innate Immunity and Sets the Stage for Adaptive Immunity

As the ischemic cascade progresses (Box 1), cell death leads to a new phase of the inflammatory response (Figure 2). Dying and dead cells release “danger signals” that activate the immune system. Some of these signals, like the nucleotides ATP and UTP, are released by cells under stress when the cell membrane is still intact and set the stage for the subsequent immune response [31-32].

ATP and Neurotransmitters

Extracellular ATP increases within minutes after ischemia as a result of neuronal and glial depolarization or escape through damaged plasma membranes. ATP is also released by vascular cells and blood cells, and may promote intravascular coagulation and platelet aggregation. High parenchymal ATP levels activate P2×7 receptors in microglia leading to release of pro-inflammatory mediators (Figure 2). Activated microglia develop many characteristics of macrophages including ameboid morphology, migratory capacity, phagocytosis and MHC class II-restricted antigen presentation (Box 3). At the same time, neurotransmitters release after I/R may counteract the emerging inflammatory response [33-35].

Microglia express a wide variety of neurotransmitter receptors, including AMPA, kainate, adrenergic, GABAB opioid, and cannabinoid receptors. With some exceptions activation of these receptors downregulates microglial cytokine, ROS and NO production, and suppresses the secretory response in mast cells [36-38]. Therefore, ATP represents an early neuronal danger signal, promoting the inflammatory response of resident immune cells, whereas neurotransmitter release may oppose these changes and counteract inflammation.

Box 1: From ischemia to infarction: The ischemic cascade

The brain is critically dependent on the continuous delivery of oxygen and glucose through blood flow, and interruption of the cerebral blood supply leads to irretrievable brain damage [Moskowitz MA et al 2010]. Ischemic damage results from a cascade of cellular and molecular events triggered by sudden lack of blood flow and subsequent reperfusion of the ischemic territory. Neurons are more vulnerable than glia and vascular cells, and when exposed to hypoxia-ischemia quickly become dysfunctional and die [Lipton P. et al 1999]. In ischemia produced by occlusion of the middle cerebral artery, the most common type of stroke, the damage is more rapid and severe in the center of the ischemic territory (ischemic core), where flow is lowest. At the periphery of the ischemic region, the so called ischemic penumbra, neuronal damage develops more slowly because blood flow arising from adjacent vascular territories (collateral flow) keeps cerebral perfusion above the threshold for immediate cell death [Moskowitz MA et al 2010]. In the ischemic core the major mechanism of cell death is energy failure. Without oxygen and glucose neurons cannot generate the ATP needed to fuel the ionic pumps that maintain the ionic gradient across the neuronal membrane, mainly the Na^+/K^+ ATPase [Lipton P. et al 1999]. Consequently, massive Na^+ and Ca^{2+} cytoplasmic accumulation leads to swelling and degeneration of the organelles, loss of membrane integrity and dissolution of the cell (necrotic cell death). In the ischemic penumbra, the flow reduction is not sufficient to cause energy failure, and neurons remain viable for a prolonged period of time after the insult. However, neurons are stressed and critically vulnerable to pathogenic events that may tip over their fragile metabolic balance. Excessive extracellular accumulation of glutamate is a major factor contributing to the demise of the ischemic penumbra. The resulting overactivation of the NMDA subtype of glutamate receptors leads to cytoplasmic accumulation of Ca^{2+} , which activates Ca^{2+} dependent enzymes, including the proteases calpain and caspase, and enzymes producing nitric oxide, free radicals and arachidonic acid metabolites. These events lead to necrosis or programmed cell death depending on the intensity of the insult and the metabolic state of the neurons [Lipton P. et al 1999]. Injured and dying cells play a key role in post-ischemic inflammation because they release “danger signals” which activate the immune system (see text).

The mechanisms of ischemic stroke have been studied using in vitro and in vivo models of cerebral ischemia. These models do not recapitulate all the features of human stroke and may introduce important confounders, such as anesthesia and surgical trauma. Furthermore, differences in vascularization and immune responses in the human brain (Box 2) may alter the initiation and evolution of the tissue damage. However, the core features of stroke pathophysiology are comparable between animals and humans and animal models of cerebral ischemia remain a mainstay of preclinical stroke research [Bacigaluppi M et al 2010]

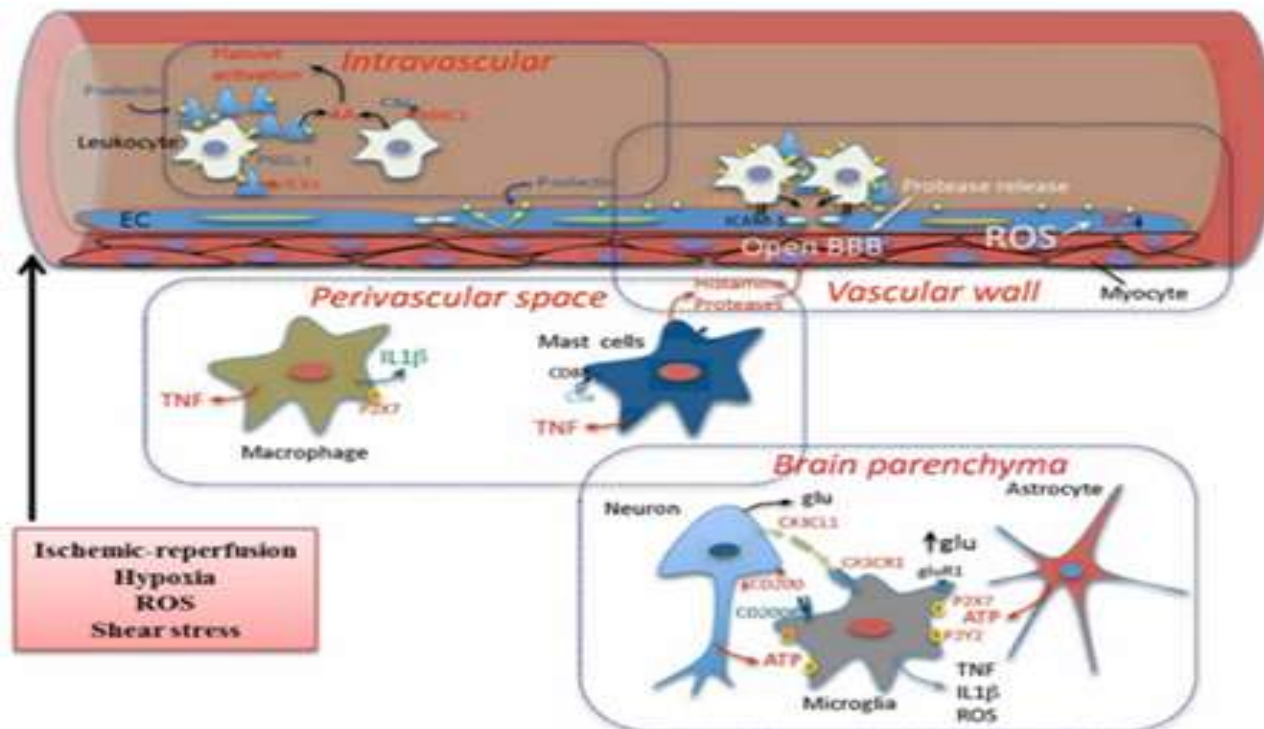


Figure 1. Early vascular, perivascular and parenchymal events triggered by I/R Hypoxia, ROS and changes in shear stress initiate the cellular events induced by I/R. In the vessels lumen, I/R leads to blood clotting, platelet aggregation and cytokine release (IL-1 α). Translocation of P-selectin on the surface of platelets and EC leads to platelet-leukocyte aggregation. Complement is activated and arachidonic acid metabolites are released. In the vascular wall, upregulation of E- and P-selectin on EC provides a platform for low affinity leukocyte binding through interaction with sialyl Le^x moieties of glycoproteins expressed on leukocytes, e.g., PSGL-1. Firm adhesion is obtained after endothelial expression of ICAM-1 interacting with leukocyte β 2 integrins (LFA-1 and Mac-1. Loss of NO promotes vasoconstriction and enhances leukocyte and platelet aggregation. MMP activation could lead to BBB breakdown and matrix proteolysis facilitating leukocyte extravasation. In the perivascular space, chemotactic complement subunits (C5a) acting on mast cell complement receptors (CD88) leads to degranulation and release of histamine and proteases, contributing to BBB leakiness. Cytokines (TNF, IL-1 β) are produced by mast cells and perivascular macrophages, providing further signals to guide leukocyte migration across the vessel wall. In the brain parenchyma, injured cells release purines (ATP), which act as early proinflammatory signals leading to production of cytokines and chemokines. Disruption of neuronal-microglial interaction (CX3CL1, CD200) and increases in extracellular glutamate (glu) acting on microglial GluR1 metabotropic receptor also contribute to the pro-inflammatory milieu.

Box 2: Cells of the innate immune system

Cerebral ischemia engages both innate and adaptive immunity, the two main branches of the vertebrate immune system [Yilmaz G et al. 2006]. The innate system is germline-encoded, rapidly activated, and relies on low affinity receptors to gain wide-ranging target recognition. The adaptive system is based on high-affinity receptors, i.e., T-cell receptors and immunoglobulins, which are randomly generated by somatic mutations. In contrast to the innate system, adaptive immunity needs antigen-driven clonal cell expansion, a process that requires several days, and retains a memory of this antigen exposure [Yilmaz G et al. 2006]. Although specific cell types are predominantly associated with one of the two branches, there is considerable overlap between the role of these cells in innate and adaptive immunity. Here we describe cells predominantly associated with innate immunity. Lymphocytes are described in Box 3.

Microglia derive from the hematopoietic system and constitute the innate immune cells of the CNS [Ginhoux F, et al 2010]. Resting microglia survey the brain parenchyma through continuous extension and retraction of their processes [Nimmerjahn A, et al 2005]. Microglia contribute to post-ischemic inflammation by producing TNF, IL-1 β , ROS and other pro-inflammatory mediators. However, they also contribute to the resolution of inflammation and tissue repair by producing IL-10 and TGF- β , as well as several growth factors, including IGF-1 (Table 1).

Perivascular macrophages are confined to the space between the vascular basement membrane and the brain surface (glia limitans), and, unlike microglia, are continuously replenished by hematogenous precursors [Bechmann I, et al 2001]. Macrophages can be classified into two groups. M1 macrophages produce pro-inflammatory cytokines (IL-1 β , IL-12, IL-23 and TNF), chemokines, ROS, and NO, thus promoting a Th1 immune response (Box 3). In contrast, M2 macrophages produce anti-inflammatory cytokines (IL-10 and TGF- β), IL-1ra, and arginase [Mantovani A, et al. 2005]. After cerebral ischemia, cytokines produced by perivascular macrophages are thought to drive the infiltration of inflammatory cells [Konsman JP, et al 2007].

Mast cells (MCs) are found in meninges and cerebral blood vessels. MC granules store vasoactive substances (histamine), cytokines (TNF), anticoagulants (heparin), and proteases (tryptase, chymase, MMP2, MMP9) [Strbian D, et al. 2006]. In addition, MCs are capable of phagocytosis, antigen presentation and can modulate the adaptive immune response [Rao KN, et al. 2008].

Blood monocytes are direct precursors of tissue macrophages. At least two different monocyte populations have been described in humans and mice according to their surface markers and function. "Classical" monocytes produce the anti-inflammatory cytokine IL-10 when challenged with LPS, whereas "pro-inflammatory" monocytes produce TNF. After cerebral ischemia, inflammatory monocytes are rapidly recruited to the site of injury where they give rise to macrophages and DC [Tanaka R, et al 2003].

Dendritic cells are specialized APC and constitute the main interface between innate and adaptive immunity (Box 3). They originate from myeloid lineage through a common dendritic precursor [Geissmann F, et al 2010]. In the normal brain, DCs are associated with meninges, choroid plexus and the CSF. Cells expressing DC markers (CD11c⁺, MHC Class II⁺) appear in the brain parenchyma after focal ischemia and originate from resident, as well as blood borne cells [Reichmann G, et al 2002].

Neutrophils are secretory and phagocytic cells of the innate immune system and carry different types of cytoplasmic granules and secretory vesicles. Major pro-inflammatory molecules stored in granules and vesicles include, iNOS, NADPH oxidase, myeloperoxidase, MMP8, MMP9, elastase, and cathepsins. After cerebral ischemia neutrophils adhere to the cerebral endothelium and transmigrate into the tissue [Yilmaz G, et al 2010]. Vesicle and granule exocytosis is induced after receptor engagement, e.g. binding to E-selectin on EC, IL-8 stimulation [Borregaard N. et al 2010].

Is the immune system comparable in rodents and humans? Circulating neutrophils predominate in humans (50–70%), while lymphocytes predominate in rodents (75–90%). Several immune-related molecules, i.e., iNOS, P2 $\times$ 7, TLR2, antigen presenting proteins, co-stimulatory molecules, immunoglobulins, and chemokines, are differentially expressed in mice and humans, or present in one species and not the other [Mestas J, et al. 2004]. Human EC can present antigens to resting CD4⁺ and CD8⁺ T cells, while mice EC can only activate CD8⁺ T [Kreisel D, et al 2002]. Consequently, the immune responses initiated by vascular pathologies may differ quantitatively and qualitatively between humans and mice. The impact of these differences needs to be considered when transposing findings obtained from rodent models of cerebral ischemia to human stroke.

Cell Death and Pattern Recognition Receptors in the Post-Ischemic Brain

A different signaling landscape emerges after cells begin to die. A wide variety of molecular signals are released from the intracellular compartment or are generated by the action of lytic enzymes escaped from dead cells on matrix proteins (Figure 2). These so-called danger associated molecular pattern molecules (DAMPs) activate pattern recognition receptors (PRRs), including toll like receptors (TLRs) and scavenger receptors, widely expressed on microglia, perivascular macrophages, and brain EC [39]. DAMPs and purines act in concert to induce the expression of proinflammatory molecules in infiltrating leukocytes (Table 1) and to prime dendritic cells (DC) for antigen presentation (Boxes 2 and 3) (Figure 2). Considering the high vascular density of the brain, inflammatory mediators released from parenchymal cells are likely to feed back on the vascular and perivascular compartments to reinforce and amplify the expression of cytokines, chemokines and adhesion molecules that drive the infiltration of blood borne cells into the ischemic tissue. After neuronal death, loss of cell-cell interaction between neurons and microglia also promotes inflammatory signaling. In the normal state, microglia are kept quiescent by contact with neurons. For example, CD200, a surface protein expressed in neurons, interacts with its receptor CD200R on microglia enforcing a resting phenotype [40]. Disruption of this interaction due to postischemic loss of CD200 [41] may promote microglial activation (Figure 1). Similarly, CX3CL1 (fractalkine), a cell surface bound chemokine constitutively expressed by neurons, suppresses microglial activation through its microglial receptor CX3CR1. Thus, after neuronal injury loss of CX3CL1 results in enhanced microglial activation in several inflammatory disease models. In addition, increasing concentrations of extracellular glutamate activate metabotropic glutamate receptors on microglia leading to a proinflammatory phenotype [42-43] (Figure 1). Therefore, as neuronal death develops in the ischemic core and spreads to the penumbra the loss of the immunosuppressive effect afforded by neurotransmitter release and neuron-microglia interaction may also foster post-ischemic inflammation.

Collectively, these observations suggest that the inflammatory response after I/R starts at the vascular level, driven by non-transcriptional events triggered by hypoxia, shear stress and ROS production. As ischemia damages the brain tissue, danger signals are released first from cells under stress and then from necrotic cells. Concomitant with the loss of immunosuppressive mechanisms, these signals activate purinergic receptors and pattern recognition receptors, which induce an inflammatory response in resident brain cells and infiltrating leukocytes.

Release of nucleotides (ATP, UTP) from injured cells, including neurons, activates purinergic receptors on microglia and macrophages, and leads to production of proinflammatory cytokines. While most of these cytokines are transcriptionally induced, IL-1 β and IL-18 are processed from their pro-peptides by the activity of interleukin-1 converting enzyme (ICE; caspase 1). ICE is embedded in a multi-protein complex (NLRP3 or inflammasome) and is activated by microglial P2 $\times$ 7 receptor. Ischemic cell death leads to the formation of danger associated molecular patterns (DAMPs) molecules, which activate TLRs, especially TLR2 and 4. DAMPs released by ischemia include HMGB1, an intracellular DNA binding protein released after cellular injury, HSP60, and β -amyloid (A β), among others.

Table 1. Showing Mediators of post-ischemic inflammation and their producing cells

INITIATION non-transcriptional [cell type]	AMPLIFICATION Transcriptional [cell type]	RESOLUTION Transcriptional [cell type]
Cytokines: IL-1 β [MG, PVM, MC] IL-1 α [PLT] TNF [MC]	Cytokines: IL-1, IL-6, IL-10, IL-17, IL-20, TNF [EC, PVM, MG, AG, Neu]	Cytokines: TGF β , IL-10, IL-17, IL-23, [T cells, MG, Macr, AG]
Chemokines: CCL5 (RANTES), CXCL4, CXCL7 [PLT] CX3CL1 (Fractalkine) [Neu]	Chemokines: CCL2 (MCP-1), CCL3 (MIP- 1 α), CCL5 (RANTES), CXCL2/3 (MIP2), CXCL8 (IL-8) [EC, PVM, MG, AG, Neu]	Chemokines: NA
Adhesion molecules: P-selectin [EC, PLT]	Adhesion molecules: ICAM1, VCAM1, P-selectin, Eselectin, Mac-1, VLA-1, [EC, Leuk, PVM, MG, AG]	Adhesion molecules: BDNF, EPO, FGF, G-CSF, GDNF, HB-EGF, IGF-1, NGF, VEGF [MG, AG, PVM, Macr EC, Neu]
Proteases: Elastase, MMP8, MMP9, MT6-MMP [Leuk] Clotting factors [Circ] Complement [Circ, EC, AG Neu]	Proteases: MMP2, MMP9 [EC, Leuk] Complement [Circ, EC, AG Neu]	Proteases: MMP9 [AG, Neu] Complement [Circ, EC, AG Neu]
Small molecules: Prostanoids, Leukotrienes [EC, PLT, MG, Neu] ATP [Circ, Neu] Radicals [EC, PLT, Leuk PVM, MG, Neu]	Other: iNOS [MG, Leuk, EC] COX-2 [Neu, MG, Leuk, EC] LOX [Neu, Leuk] PTGES [Neu, MG, Leuk, EC] NADPH oxidase [MG, Leuk]	Small molecules: Cyclopentenones prostaglandins Lipoxins Docosanoids (resolvins, protectins)
Note: AG: astroglia; Circ: Plasma; EC: endothelial cells; Leuk: leukocytes; Macr: macrophages; MC: mast cells; MG: microglia; Neu: neurons; PLT: platelets; PVM: perivascular macrophages. ATP: adenosine triphosphate; BDNF: brain-derived growth factor; COX-2: cyclooxygenase-2; EPO: erythropoietin; FGF: fibroblast growth factor; G-CSF: granulocyte colony-stimulating factor; GDNF: glial cell derived neurotrophic factor; HB-EGF: heparin-binding epidermal growth factorlike growth factor; ICAM1: intercellular adhesion molecule 1; IGF-1: insulin-like growth factor 1; IL: interleukin; iNOS: inducible nitric oxide synthase; LOX: lipxygenase; Mac-1: macrophage-1 antigen; MIP: macrophage inflammatory protein; MMP: matrix metalloproteinase; NGF: nerve growth factor; PTGES: prostaglandin E2 synthase-1; RANTES: regulated upon activation, normally T-expressed, and presumably secreted; TGF β : transforming growth factor- β ; TNF: tumor necrosis factor; VCAM1: vascular adhesion molecule 1; VEGF: vascular endothelial growth factor; VLA-1: very late activation antigen-1;		

TLRs, in concert with scavenger receptors such as CD36, upregulate pro-inflammatory gene expression through the transcription factor NF- κ B30, DAMPs also derive from matrix breakdown by lytic enzymes released from dead cells and by the action of reactive oxygen species (ROS) on lipids. The cytokine production and complement activation resulting from these events leads to increased leukocyte infiltration and enhances tissue damage, which, in turn, produces more DAMPs. Antigens unveiled by tissue damage are presented to T cells, setting the stage for adaptive immunity.

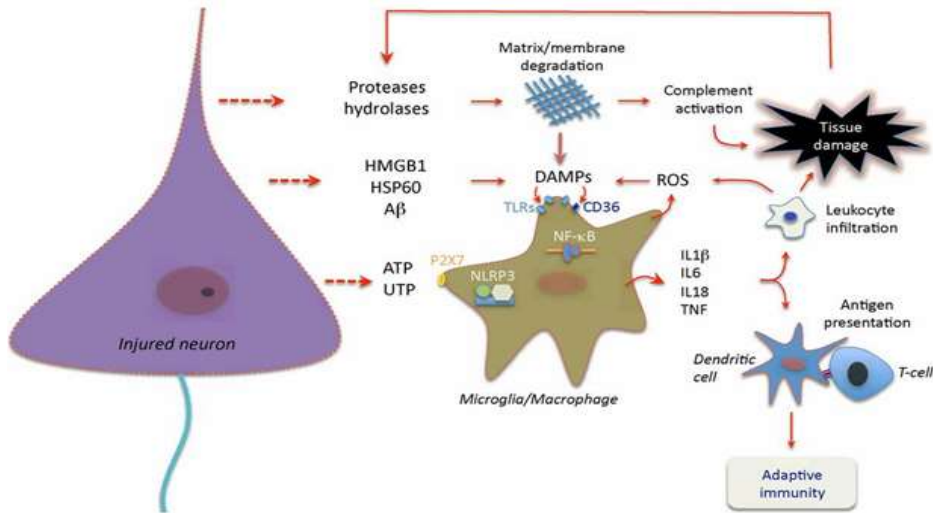


Figure 2. Cell death and activation of pattern recognition receptors set the stage adaptive immunity.

Does a Classical Adaptive Immune Response Contribute to Ischemic Brain Injury?

Danger signals released from damaged cells also promote the presentation of tissue antigens that were previously hidden by the BBB or that develop as a result of the breakdown of cell membranes (Figure 2). Antigen presentation leads to the development of cellular and humoral immunity directed against the antigens (Box 3).

This adaptive immune response has the potential of inducing autoimmunity against the organ in which the cell death occurred, as described in the heart (Dressler's syndrome), eye (sympathetic ophthalmia) and pancreas (diabetes) [44]. Furthermore, the damaging effect of adaptive immunity is well established in MS and in models of autoimmune demyelination. The next section will review the evidence for a pathogenic role of adaptive immunity in stroke.

Box 3: The spectrum of lymphocyte subtypes

Lymphocytes are key cells in innate and adaptive immune responses. B-lymphocytes (B-cells) are responsible for humoral immune responses characterized by the production of antibodies that attack and neutralize specific antigens. T-lymphocytes (T cells) are involved in cellular immunity, in which extraneous antigens are suppressed by a cytotoxic cellular response [Abbas, Ak. 2010]. T cells express the glycoprotein CD4 or CD8 on their surface, a feature that determines their function and fate.

Antigen presentation

Central to adaptive immunity is antigen presentation, which is performed by APC, mainly DC and macrophage. APC patrol the environment and when they recognize a foreign antigen, a protein for example, they degrade it into peptides, some of which are then assembled with MHC class II molecules and displayed on the APC surface. The MHC-class II-antigen complex is recognized by the T-cell receptor (TCR) of CD4+ T cells, and this interaction in the presence of appropriate co-stimulatory molecules on APC (B7-1, B7-2) and T cells (CD28) results in T-cell activation [Greenwald RJ, et al 2005]. TCRs include either α - β chains or γ - δ chains, immunoglobulin-like proteins consisting of variable and fixed regions. After antigen presentation, CD4+ T cells undergo clonal expansion in lymphoid organs, a process promoted by autocrine production of IL-2 [Abbas, Ak. 2010].

Helper T cells

With notable exceptions, CD4+ T cells become helper T cells (Th), so called because they do not have cytotoxic function, but act as “helpers” by coordinating and modulating immune responses. Th cells include effector and regulatory cells. Depending on the molecular signals present in their milieu, different subpopulations of effector Th cells can develop, each characterized by a specific pattern of cytokine production. Th type 1 (Th1) effector cells secrete IFN γ and TNF and their development is promoted by IL-12 through the transcription factor t-bet. Th1 cells stimulate innate and T-cell induced immune responses leading to cytotoxicity. Th2 cells secrete IL-4, IL-5, IL-9, IL-10, IL-13 and promote humoral immunity, mucosal immunity, and responses directed against extracellular pathogens. IL-4 promotes Th2 cell development, which is regulated by the transcription factor GATA-3. Th $_{17}$ cells secrete IL-17 and their development is driven by TGF β and IL-6, and involves the transcription factor ROR γ t. Th17 cells participate in autoimmunity but have not been implicated in cerebral ischemic damage. Regulatory T cells (T $_{reg}$ or suppressor T cells) are either present naturally, or can develop from other Th subtypes in the presence of TGF β . T $_{reg}$ development requires the transcription factor FoxP3. Unlike other Th cells, T $_{reg}$ induce immunosuppression by producing IL-10 and TGF β [Wan YY. 2010]. Therefore, T $_{reg}$ are critical for the maintenance of the homeostasis of the immune system by counterbalancing the destructive effects of excessive inflammation and may play a protective role in cerebral ischemia (also see text).

Cytotoxic T cells

In contrast to CD4+ T cells, CD8+ T cells are cytotoxic. The TCR of CD8 expressing T cells binds the antigen presented with MHC class I molecules and co-stimulatory molecules. Unlike MHC class II, MHC class I molecules are present in every cell. After clonal expansion, activated CD8+ T cells patrol the internal environment in search of somatic cells expressing the antigens against which they were sensitized. Cytotoxic T cells kill somatic cells by permeabilizing the membrane with perforin and inducing apoptosis via granzyme-induced caspase activation or the Fas ligand pathway (Box 4). Lymphocytic cells with cytotoxic function also include natural killer (NK) cells and natural killer T cells (NKT). NK cells lack a TCR and, as such, do not require antigen presentation for their activation and cytotoxicity, which is triggered by interferons or cytokines. NKT cells have a simplified TCR that recognizes glycolipids presented by cells also expressing the MHC class I-like glycoprotein CD1 [Biron CA, et al 1999]. NKT cells exert their cytotoxic effect by releasing large amounts of IFN γ , IL-2, and TNF.

$\gamma\delta$ T cells

These are a subset of effector lymphocyte with a TCR comprising $\gamma\delta$ chains and particularly enriched in mucous membranes. Like NKT, these cells recognize non-peptide antigens and react to danger signals produced by stressed cells [Bonneville M, et al 2010]. $\gamma\delta$ T cells can exert different functions depending on the context in which they operate, ranging from cytotoxicity, to antigen presentation, immunoregulation, and production of growth factors [Hayday AC. 2009]. In cerebral ischemia, $\gamma\delta$ T cells have been implicated in both cytotoxicity and protective immunomodulation (also see text).

Stroke and Adaptive Immunity

Antibodies against CNS antigens develop after ischemic stroke, suggesting a humoral immune response to the injury, and circulating T cells become sensitized against CNS antigens, such as myelin basic protein (MBP) and related peptides. APC are reduced in the periphery and increased in the ischemic brain both in rodent and human stroke. The accumulation of APC coincides with the peak of lymphocytic infiltration and is associated with expression of MHC class II molecules and the co-stimulatory molecule CD80, findings suggestive of antigen presentation (Box 3). Support for involvement of adaptive immunity also comes from studies on the role of lymphocytes in models of focal cerebral ischemia. Ischemia leads to infiltration of the major lymphocyte subtypes into the ischemic brain (Box 3). Increasing evidence suggests that lymphocytes contribute to ischemic injury (Figure 3) [45-48]. Lymphocyte-deficient mice are protected from ischemic damage. The protection has been attributed to T cells, because B-cell deficient mice or lymphocyte-deficient mice reconstituted with B-cells are still protected from injury. $\gamma\delta$ T cells also have been shown to contribute to the injury by releasing the proinflammatory cytokine IL-17 (Figure 3). In contrast, T regs are protective in the late stage of cerebral ischemia, an effect evident only if the injury is small [49-51].

Additional evidence in favor of the involvement of cell-based adaptive mechanisms in stroke damage was provided by studies in which animals were tolerized against myelin-derived peptides (Table 2). Repeated mucosal administration of myelin antigens (tolerization) prior to arterial occlusion protects rodents from ischemic brain injury. Although tolerization is antigen specific, its beneficial effects are not restricted to immune responses directed at the inducing antigen, but are more widespread, a phenomenon termed bystander suppression [52].

The protection can be induced in naïve mice by adoptive transfer of splenocytes or CD4⁺ T cells from tolerized animals suggesting the involvement of cellular immune mechanisms [53]. Examination of T-cell function indicates that activation of tolerized T cells by the antigen unveiled by the stroke induces a Th2 cytokine response (Box 3). The effect has been attributed to IL-4 and IL-10 production that favors the formation of TGF β secreting Treg cells [54-56].

Other studies have found that administration of recombinant T-cell receptor ligand (RTL), consisting of α 1 and β 1 domains of MHC class II complex bound to a myelin peptide antigen (MOG35-55), reduces stroke volume in focal ischemia. The protective effect is associated with reduction of infiltrating inflammatory cells and may result from suppression of autoreactive T cells targeted against myelin. Although RTLs may not bind T cells, but APC and platelets, the findings suggest a role of cellular immunity in the mechanisms of ischemic brain injury [57-58].

Despite the evidence supporting an autoimmune response against the post-ischemic brain, there are inconsistencies with the hypothesis that classical adaptive immunity contributes to ischemic brain injury. The temporal profile of the involvement of T cells in brain damage is not consistent with established concepts of adaptive immunity (Figure 3). Thus, the protective effect observed in lymphocyte deficient mice or afforded by blocking postischemic trafficking of T cells into the ischemic brain occurs 24-48 hrs after ischemia, whereas adaptive responses require an interval of 7-10 days from antigen presentation to the clonal expansion of autoreactive T cells and immune attack on the target organ (Box 3) [59].

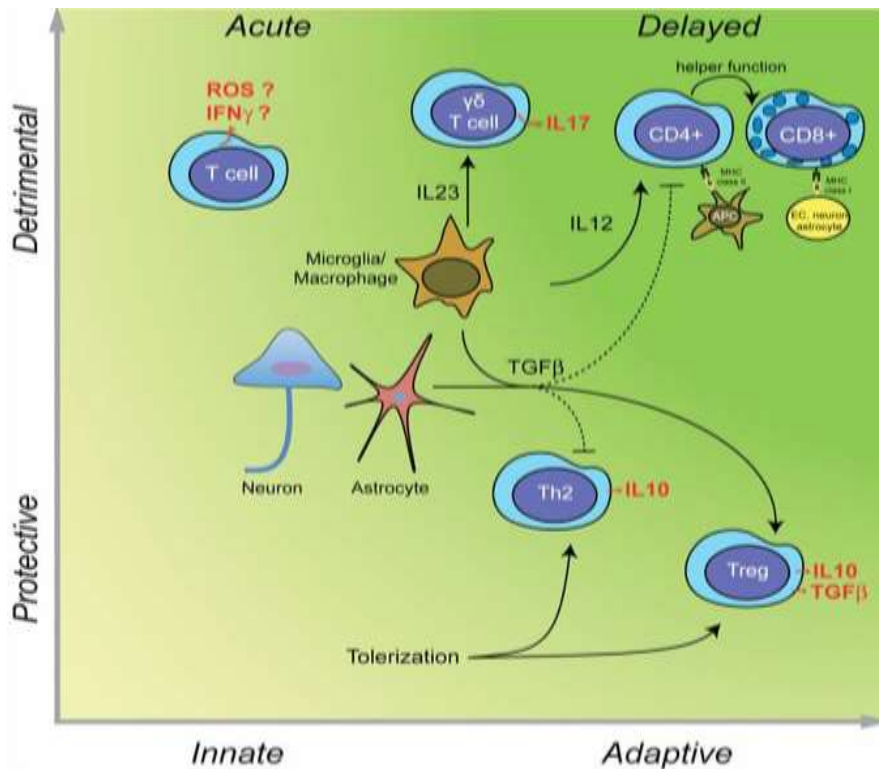


Figure 3. Deleterious and beneficial roles of T cells in stroke.

In the acute phase of cerebral ischemia, unprimed T cells contribute to tissue damage in an antigen independent manner (innate immunity), possibly through IFN γ and ROS (left upper quadrant). $\gamma\delta$ T cells, activated by IL-23 released from microglia/macrophages, produce the cytotoxic cytokine IL-17 and contributes to acute ischemic brain injury⁴¹. However, T cells can also be protective. TGF β produced by neurons, glia, or macroglia/macrophages promotes the development of Treg cells secreting the protective cytokine IL-10 and inhibits Th1 and Th2 responses. Treg cells are protective in models of cerebral ischemia. Induction of mucosal tolerance with CNS antigens produces an adaptive response, which leads to the establishment of autoreactive Th2 cells producing IL-10 and Treg cells producing IL-10 and TGF β ¹⁰⁷, is highly protective in experimental stroke (right lower quadrant). As discussed in the text, there is no evidence that adaptive immunity contributes to acute ischemic brain injury. However, weeks and months after stroke, autoreactive CD4⁺ and CD8⁺ T cells targeting CNS antigens could develop (right upper quadrant). The resulting cell death could play a role in the delayed brain damage and atrophy that occurs after stroke.

Furthermore, reconstitution of lymphocyte deficient mice with T cells targeting non-CNS antigens worsens ischemic damage, and mice lacking co-stimulatory molecules essential for antigen-specific T-cell response are not protected from ischemia. It is also surprising that unlike autoimmune responses in other organs, where there is a prevalence for either T-helper or T-effector cell participation, both CD4⁺ and CD8⁺ T cells are involved in ischemic injury [60]. Collectively, these observations argue against an autoimmune attack on the brain resulting from presentation of CNS antigen released by the stroke.

Box 4: How does inflammation work to kill?

ROS, NO, the complement system, apoptosis inducing receptors, and the perforin pathway represent the major effectors of cell death in inflammation.

ROS and NO

In the setting of inflammation, O_2^- is produced mainly by NADPH oxidase, an enzyme expressed in virtually all inflammatory cells (see box 2 and 3). At the same time, large amounts of NO are produced by de novo expression of iNOS. NO reacts preferentially with O_2^- to form the cytotoxic molecule peroxynitrite. H_2O_2 is derived from O_2^- dismutation and gives rise to the highly toxic hydroxyl radicals via the Haber-Weiss reaction, facilitated by the increased availability of free iron in ischemia [Selim MH, et al 2004]. These toxic molecules alter cellular proteins, lipids, and ribonucleic acids leading to cell dysfunction or death and have been implicated in the tissue damage produced by post-ischemic inflammation. For example, suppression of NADPH oxidase or iNOS activity protects the brain in the late phase of cerebral ischemia.

The complement system

The complement system is a proteolytic cascade composed of different subunits (C1 through C9) that leads to cell lysis via the formation of a pore like structure assembled from C9 termed the membrane attack complex (MAC). Complement also enhances phagocytosis (opsonization), or acts as a chemotactic-activating stimulus for inflammatory cells. The classical pathway for complement activation involves the interaction of the C1q subunits with immune complexes or IgM to activate the cascade. The lectin pathway utilizes mannose and N-acetylglucosamine-binding lectin to activate subunits C2 and C3, while the alternative pathway amplifies the C3 activity generated by the other pathways to form the MAC. After ischemia, complement is activated and its components are either upregulated in glia and neurons, or enter the brain through BBB breakdown [Yanamadala V, et al 2010]. The involvement of complement in ischemic damage is indicated by the fact that deletion of endogenous complement inhibitors increases brain injury, whereas their upregulation or administration is protective.

Apoptosis inducing receptors (Fas and TRAIL)

Fas (CD95), a member of the TNF-receptor superfamily, is expressed on neurons and glia after ischemia [Rosenbaum DM, et al. 2000], while its ligand FasL (CD95L) is present in neurons, microglia, cytotoxic T cells (CTL), $\gamma\delta$ T cells and NK cells [Strasser A, et al. 2009]. Fas ligation results in formation of the death inducing signaling complex (DISC) and subsequent activation of caspase-8 and of the pro-apoptotic factor Bid. Similarly, the TNF-related apoptosis-inducing ligand (TRAIL) is expressed de novo after ischemia in astrocytes and microglia, and induces apoptosis by engaging its receptors on neurons and glia. This pathway is involved in ischemic cell death because TRAIL inhibition or Fas mutation ameliorates ischemic injury [Cui M, et al. 2010 and Martin-Villalba A, et al 1999].

Perforin/Granzyme

This pathway is utilized by CTL, NKT, and NK cells and usually requires molecular recognition associated with MHC class I or class I-like molecules. After engagement of accessory surface receptors, e.g., ICAM-1, degranulation occurs releasing the protease granzyme, perforin - homologous to the complement subunit C9 - and the proteoglycan serglycin. This complex is internalized by the target cell, and granzyme triggers apoptosis by activating caspase-3 and Bid. The involvement of this pathway in ischemic brain injury is suggested by the observations that genetic deletion of perforin is protective [Liesz A, et al 2011].

The Lymphocyte Puzzle

If lymphocytes do not mediate an autoimmune attack on the brain how do they contribute to the early phase of ischemic brain injury? One possibility is that these cells participate in the cerebrovascular dysfunction occurring after ischemia or have prothrombotic actions leading to microvascular occlusions [61]. These effects would be deleterious by preventing reperfusion and by compromising collateral flow after I/R. However, lack of lymphocytes does not improve post-ischemic cerebral blood flow at least in the acute phase [62], nor does it suppress thrombus formation [50]. Therefore, effects of lymphocytes altering microvascular perfusion or patency seem unlikely. Another possibility is that NKT or $\gamma\delta$ T cells, T cells that have a simplified TCR and may not require antigen processing and MHC presentation, as well as NK cells (Box 3), are responsible for these early cytotoxic effects of lymphocytes. In support of this hypothesis, NKT cells are not present in Rag1^{-/-} mice and SCID mice [63] and they could contribute to explain the early neuroprotection observed in these models of lymphocyte deficiency [49-50]. However, CD1-deficient mice lack NKT cells and are not protected from ischemic injury at 24 hrs [50], suggesting that NKT cells may not be involved in the early phase of the injury. $\gamma\delta$ T cells have been implicated in ischemic brain injury, but their involvement seems restricted to the late phase of cerebral ischemia (4 days) [50-51].

Considering the limited number of studies available, the involvement of NK, NKT and $\gamma\delta$ T cells, lymphocyte subtypes that act in a fashion akin to innate immunity, needs further exploration. Collectively, these data suggest that, although an antigen specific immune response may develop following stroke, evidence that autoreactive T cells attack brain antigens exposed by ischemic damage against which they were sensitized is lacking. Lymphocytes do play a role in the development and progression of the injury, but the mechanism of their powerful effect does not conform to the tenets of classical autoimmunity. The role of NK, NKT and $\gamma\delta$ T cells, which could contribute to the acute phase of the injury, needs further exploration. Similarly, considering the evidence for humoral immune responses in stroke, the contribution of B-cells to the damage needs a more in depth assessment.

Resolution of Inflammation and Tissue Repair

Post-ischemic inflammation is a self-limiting process that eventually subsides and prepares the terrain for the structural and functional reorganization of the injured brain. The factors governing resolution of inflammation and the reestablishment of tissue homeostasis are still poorly understood, particularly in brain. Increasing evidence suggest that resolution of inflammation is not a passive process due to exhaustion of the signaling, but is orchestrated by the interplay of a large number of mediators which actively suppress the inflammatory response. Major steps in process include removal of dead cells, development of an anti-inflammatory milieu, and generation of pro-survival factors fostering tissue reconstruction and repair [64-65].

Clearing Dead Cells

Microglia and infiltrating macrophages constitute the predominant phagocytes which remove dead cells and tissue debris after stroke, a process orchestrated by “find me” and “eat me” signals. “Find-me signals”, including purines released from injured cells and chemokines, attract microglia and macrophages to the site of injury. These phagocytic cells are then presented with “eat-me signals” associated with dying or dead cells (Figure 4) [66-69].

TGF β , IL-10 and the Anti-Inflammatory Milieu

TGF β and IL-10 are pleiotropic immunoregulatory cytokines that play a crucial role in the development of the anti-inflammatory milieu associated with tissue repair (Figure 4). The production of these cytokines is promoted by phagocytosis and occurs in concert with the removal of dead cells [72]. TGF β is upregulated after ischemia primarily in microglia and macrophages and, in addition to its neuroprotective properties, has also profound effects on immune cells. Although well known for its proinflammatory effects, TGF β can suppress inflammation by inhibiting Th1 and Th2 responses and promoting Treg cell development [59]. Similarly, the immunoregulatory cytokine IL-10, produced by different cells including Treg cells [42], has both neuroprotective and anti-inflammatory activities (Figure 4). Therefore, postischemic production of TGF β and IL-10 can facilitate tissue repair by promoting the resolution of inflammation and exerting direct cytoprotective effects on surviving cells in the ischemic territory.

Growth Factors

Post-ischemic production of growth factors helps to establish an environment that is favorable to neuronal sprouting, neurogenesis, angiogenesis, gliogenesis and matrix reorganization [60]. Inflammatory cells, as well as neurons and astrocytes, are capable of producing a vast array of growth factors (Table 1). For example, microglia are required for the full expression of IGF-167, a critical factor in post-ischemic neuronal sprouting, whereas reactive astrocytes are required for functional recovery after stroke [73-75]. Vascular endothelial growth factor (VEGF), a key growth factor in post-ischemic angiogenesis, is produced by reactive astrocytes, and its action may require neutrophil MMPs, suggesting a link between inflammatory cells and angiogenesis [76-77]. However, VEGF administration early after ischemia or in excessive doses may enhance the damage [78-79]. The role that inflammatory signaling in brain recovery has also been highlighted by studies in which the transcriptome of sprouting neurons was defined indicating involvement of MHC I class molecules and complement subunits [74].

The evidence presented above indicates that cells of the immune system serve a fundamental role in all the phases of post-ischemic brain recovery. But, the limited data available provide only a glimpse into the complex sequence of events that reestablish the structural and functional homeostasis of the brain after stroke. Additional investigations on recently identified mediators instrumental to inflammation resolution and tissue repair, such

as lipoxins, resolvins, protectins, progranulins, and cyclopentenone prostaglandins (Table 1) (Figure 4), are needed to fully elucidate the role of the immune system in brain repair after stroke [64-65].

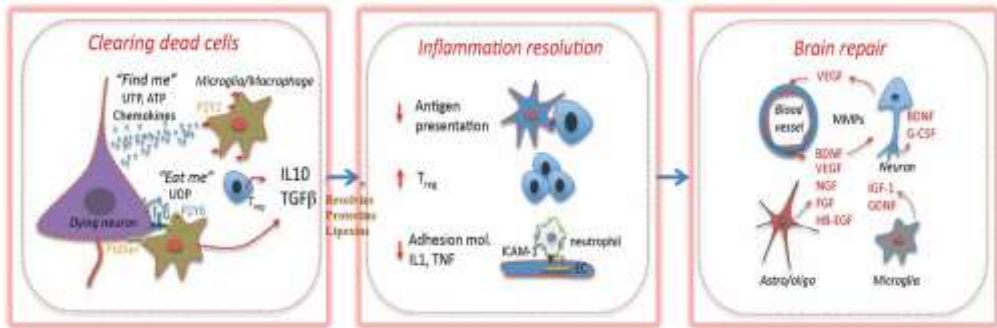


Figure 4. Resolution of inflammation and tissue repair.

Clearing of dead cells and suppression of inflammation are key events in brain repair. “Findme signals” (UTP, ATP) attract microglia and macrophages through P2Y2 receptors. “Eatme signals” include UDP, which act on P2Y6 receptors to stimulate microglial phagocytosis, and phosphatidylserine (PtdSer), which is translocated to the outer leaflet of the plasma membrane of apoptotic cells. PtdSer binding proteins involved in the clearance of dead cells include MGF-E8 on microglia and TIM4 on macrophages. Immunoglobulins directed against CNS antigens, which appear after stroke, may also promote phagocytosis by engaging Fc receptors on phagocytic cells. Phagocytosis promotes secretion of IL-10 and TGFβ56, which, in turn, suppress antigen presentation, promote Treg formation, inhibit expression of adhesion molecules in EC and production of proinflammatory cytokines. TGFβ and IL-10 are also neuroprotective¹, and may facilitate brain repair processes. In addition, arachidonic and omega-3 fatty acids metabolites lipoxins, resolvins, and protectins, which play an active role in the resolution of inflammation in other organs, could also contribute to suppress post-ischemic inflammation. Growth factors and MMPs produced by EC, neurons, astrocytes, oligodendrocytes and microglia are critical molecules driving tissue reorganization and repair.

Stroke and Systemic Immunity

Concomitant with the inflammatory response involving the brain, immunological changes are also observed in the blood, bone marrow, spleen and other lymphoid organs. Genome profiling of peripheral blood in stroke patients has demonstrated characteristic patterns of inflammatory gene expression that can help determine the cause of ischemic stroke, reflecting the specificity of the systemic inflammatory response to brain injury [80-81]. In rodent models as in patients with ischemic stroke, white blood cell count and expression of cytokines and inflammatory markers are increased within hours after ischemia. Such acute phase response is followed by a dramatic immunodepression, especially in patients with large strokes, characterized by lymphopenia, reduced functional activity of monocytes, upregulation of anti-inflammatory cytokines, lymphocyte apoptosis and splenic atrophy [82-84]. These immunological changes are associated with an increased tendency to respiratory and urinary tract infections, which are responsible for considerable morbidity and mortality in stroke patients. Infections tend to occur in patients with larger stroke and with low CD4 lymphocyte counts and elevated levels of IL-10 and IL-6, reflecting immunodepression [85].

How these systemic immune changes are mediated is not completely understood, but there is evidence that sympathetic activation and the attendant release of stress steroids and catecholamines are involved. Thus, cortisol and catecholamines are elevated in stroke patients most susceptible to infection, and steroid antagonists and the β -adrenergic receptor antagonist propranolol counteract post-stroke lymphocyte apoptosis and infection propensity in rodent models [86-87].

Bright and Dark Sides of Post-Stroke Immunosuppression

What is the biological significance of post-stroke immunodepression? One possibility is that the lymphopenia and immunosuppression limit the development of autoreactive T cells targeted to CNS antigens and dampen a potential autoimmune attack on the brain [88-89]. As discussed in the previous section the relative rapidity of the development of ischemic brain injury is not consistent with the temporal profile of an adaptive response against the brain.

However, it is conceivable that sensitization to CNS antigens plays role in the long-term outcome of the stroke (Figure 3). About 30% of stroke survivors have dementia, often associated with brain atrophy. The bases for the cerebral atrophy are not entirely clear, but immunological mechanisms triggered by the stroke cannot be discounted. Pathological studies have shown an inflammatory infiltrate that persists for years after the stroke.. However, these focal inflammatory changes have not been linked to poststroke immunosuppression or dementia, and their pathogenic significance remains uncertain. On the other hand, post-stroke immunosuppression is deleterious in that it increases the incidence of infections, a major determinant of poor neurological outcome, morbidity and mortality [88-89]. Acute infection could also negatively affect stroke outcome by upregulating costimulatory molecules and promoting antigen presentation. This possibility is supported by studies in which bacterial lipopolysaccharide (LPS) administered at the time of reperfusion to simulate post-stroke infection, worsens the outcome of experimental stroke and increases post-ischemic brain atrophy assessed 1 month after stroke. The effect is associated with increased expression of B7.1(CD80), a co-stimulatory molecule needed for efficient antigen presentation (Box 3), as well as T-cell sensitization against CNS antigens and a Th1 cytokine response [90-92]. Therefore, post-stroke immunosuppression is detrimental by increasing the incidence of systemic infections and, possibly, by promoting antigen presentation and autoimmunity against the brain, which may play a role in the long-term sequelae of the stroke. At the same time, immunosuppression could be beneficial by attenuating such delayed autoimmune response.

Bench to Bedside: Trials, Tribulations and Therapeutic Opportunities

Ischemic stroke remains an enormous therapeutic challenge. Currently, thrombolysis with tissue plasminogen activator (tPA) is the only effective therapy, but due to the narrow therapeutic window of less than 4.5 hrs and safety concerns, fewer than 5% of stroke patients receive this treatment (87). Among the potential therapeutic approaches targeting the ischemic cascade (Box 1), preclinical studies in rodent models suggest that suppression of inflammation offers unique advantages. First, these treatments have an extended therapeutic

window and are effective when administered up to 12–24 hrs after stroke [93]. Therefore, they could be used in patients who fail the time window for thrombolysis. Second, because suppression of inflammation is also beneficial in models of cerebral hemorrhage (90), concerns about worsening brain injury in patients in whom hemorrhagic stroke has not been excluded would be minimized and early treatment by emergency medical teams would become feasible. Finally, considering that the inflammation particularly deleterious in ischemia associated with reperfusion [94], suppression of inflammation would be a fitting complement to reperfusion therapy using thrombolytics or intravascular clot removal. Although these considerations are based on animal models, which may not recapitulate full in human disease (Box 1), inflammation is a critical pathogenic component of human stroke and remains an attractive target for therapeutic intervention.

Anti-Inflammatory Agents

Blocking antibodies directed against adhesion molecules (ICAM-1, MAC-1), or recombinant neutrophil inhibitory factor have not been effective in clinical trials. In the case of the ICAM-1 trial, the negative outcome has been attributed to deleterious immunoactivation resulting from administration of a mouse antibody to humans, as reproduced in an experimental study in which murine antibodies to rat ICAM-1 were administered to rats [95-96]. Although there also might be other reasons for these failures, a likely contributing factor is that post-ischemic inflammation acts through multiple redundant pathways that cannot be effectively suppressed by blocking a single cytokine or adhesion molecule, as attempted in these clinical trials. Thus, neutralizing upstream mediators of the signaling cascade or blocking multiple inflammatory pathways would be more effective. For example, blocking upstream components of inflammatory signaling, such as complement, TLR or scavenger receptors is highly protective in experimental models. Furthermore, minocycline, an agent with multiple neuroprotective actions including broad anti-inflammatory properties, has shown promise in clinical trials [97]. Another strategy, described in the next section, is to develop approaches in which the immune system is directed to suppress the deleterious effects of inflammation while enhancing its protective potential.

Immunomodulation and T-Cell Based Approaches

Ischemic tolerance provides an example of protective immunomodulation. Ischemic tolerance or preconditioning is a phenomenon in which a sub-lethal injurious stimulus protects an organ against a subsequent lethal stimulus. For example, a short non-damaging ischemic insult to the brain (local preconditioning) or other organs (remote preconditioning) protects the brain from a subsequent damaging ischemic insult [98-99]. Similarly, administration of low doses of LPS protects the brain from ischemic damage. Although ischemic tolerance is well known to protect the brain by simultaneously suppressing multiple pathways in the ischemic cascade [99], modulation of the post-ischemic immune response has emerged as one of its key effector mechanisms [100]. In the tolerance induced by LPS, post-ischemic TLR4 signaling is redirected towards production of IFN β , which, in turn,

reprograms the immune system to suppress the production of pro-inflammatory cytokines and the infiltration of inflammatory cells [101]. On the other hand, NF- κ B-dependent inflammatory mediators, such as IL-1, TNF, iNOS-derived NO and ROS, are also required for the full expression of the tolerance [99–102], indicating that the protection does not rely just on the suppression of deleterious inflammatory mediators, but on a fine balance between pro- and anti-inflammatory signaling. One of the challenges, therefore, is to learn how to modulate the immune system to replicate the beneficial inflammatory milieu induced by preconditioning. Tolerization may provide the opportunity to achieve this goal. Induction of immune tolerance through mucosal exposure to myelin antigens or E-selectin promotes a protective Th2 response, which acts through multiple pathways to suppress the deleterious effects of inflammation [55]. Due to the need to establish tolerization prior to injury, this approach, like preconditioning, would be more appropriate for stroke prevention in high-risk patients than acute stroke treatment. Another strategy is based on administration of RTL. As discussed in the section on adaptive immunity, RTL suppresses the infiltration of inflammatory cells and provides neuroprotection even if administered after the onset of cerebral ischemia [57]. Similarly, administration of the immunomodulatory co-polymer Poly-YE ameliorates neurological function without reducing injury volume, an effect attributed to increased production of growth factors and hippocampal neurogenesis [104]. The full translational potential of treatments based on immunomodulation has not been established. For example, therapeutic window, efficacy in females and in aging, protection in the presence of cardiovascular risk factors and efficacy in higher order species need further exploration. Nevertheless, its powerful protective effects justify additional investigations in this direction.

The recent identification of IL-17 secreting T cells as a critical effector of the tissue damage in autoimmune diseases (Th17 cells) and cerebral ischemia ($\gamma\delta$ T cells) [51] raises the possibility that counteracting IL-17 could be beneficial in cerebral ischemia as in experimental allergic encephalomyelitis [105]. Boosting the protective roles of Treg could also be beneficial [42], although a destructive role of these cells has also been proposed [106]. These approaches would be desirable because they target the delayed phase of the injury and are anticipated to have a particularly wide therapeutic window. However, as noted above the role of lymphocytes in ischemic injury is poorly understood and the full implications of suppressing the action of specific T and B-cell populations remain to be defined.

Fighting Inflammation: A Double-Edged Sword?

As discussed earlier in this chapter, immune cells and inflammation play an important role in tissue repair and reorganization. These beneficial effects have to be considered in developing therapeutic approaches based on restraining post-ischemic inflammation. The concern is that counteracting the inflammatory response to ischemic injury may ameliorate the tissue damage in the acute phase, but it may compromise repair mechanisms and worsen the long-term outcome of the injury. Due to the paucity of experimental studies in the recovery phase, there is no definitive experimental evidence that anti-inflammatory treatments interfere with repair processes in the post-ischemic brain. However, pro-survival effects of immune cells stemming from growth factor production, neurogenesis and neuroplasticity are well-established [106]. The essential role of inflammation in tissue repair highlights the difficulties with approaches based on full-blown suppression of inflammation.

Furthermore, in light of stroke-induced immunosuppression, the infectious complications of therapies suppressing inflammation also need to be taken into account. Therapies based on immunomodulation, in which the overall immune response is deviated from a Th1 to a Th2-type response, also have a dark side. In models of MS, tolerization with myelin antigens induces a protective Th2 response in the acute phase, but, in the long term, such Th2 response promotes B-cell differentiation and leads to a humoral attack against myelin which worsens the neurological outcome [107]. A similar worsening in the chronic phase has also been reported in tolerization applied to models of cerebral ischemia [108]. Therefore, the delayed effects of humoral immunity could counteract the shortterm benefit of suppression of cellular immunity. A more complete understanding of the immunology of stroke would enable the development of targeted approaches to selectively suppress the deleterious effects of inflammation.

The Role of Innate Immunity after Stroke

Ischemic onset is an insult for brain and immune system is firewall for the whole body. Immune system is divided into innate and adaptive systems. The innate immune system is the first line of defense, while immune system and central nervous system (CNS) were traditionally regarded as two distinct entities [109]. The existence of blood-brain barrier and absence of cerebral lymphatic vessels are largely impeding the communication between brain tissue and circulating immune cells and the antigen presenting T cells [110]. However, mounting evidence is challenging this viewpoint, indicating that ischemic stroke is complicated by mutual interplay between CNS and immune system. Innate immune response plays a dual role in stroke, exerting beneficial as well as deleterious effects on the outcome [111]. Considering the Yin and Yang effects of innate immune system, an overall suppression or activation of innate immunity might not be beneficial, while the true challenge is to selectively inhibit the deleterious effects without compromising the beneficial roles of innate immune response in tissue repair, remodeling, and recovery. It means that we should use immune system after stroke in the right time and right place.

Innate Immune System

Immune system monitors and preserves the homeostasis of CNS under normal and pathological conditions. Immune system consists of two mechanisms: the innate and adaptive immunity. The former reacts rapidly after ischemic insult and represents first step of inflammatory cascade [112], while the latter depends on antigen presentation and takes days to get activated. Therefore, innate immunity lays the foundation of the adaptive response and plays the key role in the integrated immune response secondary to cerebral ischemia. The innate immune system in the brain relies on various immune cells including resident cells such as microglia and endothelia, as well as circulating immune cells from blood such as neutrophils, monocytes/macrophages, and dendritic cells, among which microglia, neutrophils, and monocytes/macrophages are most investigated. Besides the cellular component, cytokines are also involved, mainly including interleukin- 1β (IL- 1β), interleukin-6 (IL-6), and tumor necrosis factor- α (TNF- α). Produced by immune cells, these cytokines

function to promote as well as quell inflammation, exerting both deleterious and protective roles. Immediately after cerebral ischemic onset, dying and dead neurons begin to release the so-called damage associated molecular patterns (DAMPs). Innate immune system senses the DAMPs via broad-specificity receptors and responds to the cerebral ischemic injuries within minutes, and the response remains predominant throughout the first few hours to induce postischemic inflammatory cascade (Table 2) [113].

Under normal condition, the blood-brain barrier forms a nature obstacle to prevent the entrance of circulating immune cells into the brain. However, under ischemic condition, necrotic tissues would cause local inflammation, leading to the release of inflammatory mediators like cytokines, chemokine, nitric oxide, and reactive oxygen species, which eventually leads to dysfunction of the blood-brain barrier, allowing the translocation of circulating immune cells [114].

Table 2. Showing activation and function of innate immune cells

Cell type	Activation time	Functions
Microglia	Within minutes	M1 phenotype promotes inflammation by secreting cytokines like IL-1 β , TNF- α and presenting antigens to T cell; M2 phenotype quells inflammation by secreting cytokines like IL-10, TGF- β , and tissue repair
Neutrophils	30 minutes	N1 phenotype exacerbates the damage, whereas N2 is protective
Dendritic cells	1 hour	Generally deleterious, suggested by researches to date
Macrophages	2 days	View point 1: M1 phenotype promotes inflammation and M2 phenotype quells inflammation (similar with microglia). View point 2: macrophages originating from peripheral monocytes are cytotoxic, while those from microglia are protective

The Role of Innate Immune System in Stroke

Initiation of Innate Immune Response: DAMPs and Pattern

Recognition Receptors

Neurons are particularly vulnerable to ischemic insult. Shortly after cerebral vascular accident, local ischemia would lead to the destruction of neurons in the ischemic core and peri-infarct zone, resulting in the release of various DAMPs including high mobility group protein B1 (HMGB1), uric acid, heat shock proteins, S100 proteins, DNA, and RNA, which attract and activate neighboring microglia and thereby trigger the postischemic inflammatory cascade. In spite of the mounting studies on DAMPs [115–116], it is still controversial which molecule represents the most important mediator that triggers the activation of innate immunity. Among all these DAMPs, high mobility group box 1 (HMGB1) stands out as the most investigated molecule [117]. HMGB1 is a nuclear protein normally localized in cell nuclei under the normal condition. Upon ischemia onset, however, neuronal necrosis causes the protein to translocate into the cytosol and then to passively enter the extracellular compartment. In clinical studies, Schulze and colleagues [115] detected an elevated plasma

HMGB1 level in patients with acute ischemic stroke and verified a correlation between HMGB1 level and circulating leukocytes. Release of HMGB1 by necrotic neurons in early stage of cerebral ischemia exhibits proinflammatory activity and amplifies inflammatory damage to brain tissue. Whereas intravenous injection of anti-HMGB1 monoclonal antibody would remarkably ameliorate brain infarction in middle cerebral artery occlusion models. Moreover, via electron microscopic observation, Zhang and colleagues [118] directly demonstrated that HMGB1 release induced rapid and drastic disruption of the BBB, followed by significant cerebral edema, which appeared to be in consistence with their findings by MRI. Interestingly, Hayakawa and colleagues [116, 119] found that, during the stage of stroke recovery, HMGB1 mediated beneficial plasticity and enhanced stem and progenitor cell recruitment, proliferation, and differentiation within damaged brain tissue.

HMGB1, as well as other DAMPs, is reported to induce downstream biological effects via interactions with pattern recognition receptors, including Toll-like receptors (TLRs), widely expressed on surrounding microglia, perivascular macrophages, and cerebrovascular endothelium [120]. The Toll-like receptor (TLR) pathway plays a pivotal role in the activation and amplification of innate immune response to endogenous tissue damage resulting from cerebral ischemia [121-122]. So far, 10 functional TLRs have been identified in humans as well as 12 in mice. TLR1-TLR9 are conserved in both species, while TLR 10 is not functional in mice because of a retrovirus insertion, and TLR 11, TLR 12, and TLR 13 have been lost from the human genome [123]. Among all the TLRs, TLR 2 and TLR 4 are expressed on the cell surface and detect endogenous ligands [124]. After middle cerebral artery (MCA) occlusion, TLR 2 and TLR 4 are documented to be upregulated and contribute to tissue damage by triggering the expression of inflammatory and apoptotic genes [125]. In fact, TLR 2 and TLR 4 play differential roles in acute cerebral ischemia/reperfusion injury. Hua and colleagues [126] found in genetic modified mice that TLR 4 knockout resulted in reduced infarct size, while TLR 2 knockout led to enlarged infarct size, higher mortality, and decreased neurological function, suggesting that TLR 4 contributed to cerebral ischemia/reperfusion damage, whereas TLR 2 appeared to be neuroprotective in response to cerebral ischemia. In addition to TLRs, the intracellular NOD-like receptors (NLRs) have also recently been identified as key mediators of inflammatory and immune responses [127]. NLRP 3 contributes to neurovascular damage by regulating the release of NLRP3-mediated proinflammatory mediators, and NLRP3 deficiency ameliorates cerebral ischemic injury in mice after by reducing infarcts and blood-brain barrier damage [128].

Activation of Innate Immune System

Activation of Local Resident Microglia in Central Nervous System

Microglia are the resident macrophages in brain that survey the CNS and eliminate debris via phagocytosis under normal and pathological conditions. In the resting state, microglia exhibit ramified appearance and once activated, these cells alter into an amoeboid morphology. Microglial activation is the initial step in CNS inflammation of numerous causes [129-130]. In ischemic stroke, microglia are activated within minutes of ischemic onset and microglial products are detected as early as 1 hour after stroke. Microglia express pattern recognition receptors including TLRs and NLRs to sense exogenous pathogens and endogenous danger signals [131].

Infiltration of Immune Cells from Peripheral Blood Monocytes/Macrophages Accumulation

Monocytes are resting innate immune cells derived from the blood. Upon activation, these cells would undergo morphological and functional alteration and then be referred to as macrophages. Of note, it has been controversial for years regarding the precise origin of local infiltrating macrophages [132], due to the morphological and functional similarity between activated microglia and recruited monocytes/macrophages. Once activated, microglia alter their morphology and gene expression to develop an inflammatory phenotype, making themselves indistinguishable to circulating macrophages [131]. Yet one mostly recent research has settled the debate, proving that local reactive macrophages consist of 2 distinct populations of cells, that is, a majority originates from resident microglia and a small group recruited from circulation [133]. In contrast to the immediate response of microglia, the latter group of cells is recruited no sooner than 2 days after ischemia and remains abundant through day 3 to day 7 [134].

Neutrophil Infiltration

Within the acute phase of ischemic stroke (minutes to hours), the injured tissue would release free-radicals and proinflammatory cytokines and chemokine, which would thereby upregulate adhesion molecules on endothelial cells as well as the surface of circulating immune cells, and facilitate the recruitment and migration of leukocytes [134]. Among all the components in the circulating immune system, neutrophils are the first responders [135] that are reported to react to the acute ischemia within 30 minutes and peak in the first 3 days [136]. Via neutrophil CD11b/CD18 and endothelial ICAM-1 interactions, neutrophils adhere to activated vascular endothelium and infiltrate into the injured area, and blocking of the interactions would result in reduced leukocyte accumulation [137].

Dendritic Cell Increment

As a link between the innate and adaptive arms of the immune system, dendritic cells (DCs) are key regulators in many forms of immune response [138], but the regulatory role of DCs in inflammation provoked specifically by stroke has not yet been sufficiently investigated. Kostulas and colleagues [139] stood among the first to provide data on DCs in cerebral ischemia and demonstrated ascending numbers of DCs in the ischemic hemispheres in rat models as early as 1 hour after permanent MCA occlusion. Later on, Gelderblom et al., [140] confirmed this finding by analyzing different subclasses of inflammatory cells using flow cytometric analysis and found in surprise that DCs showed one of the largest increases in cell numbers and accounted for a substantial portion among all the infiltrating immune cells with 20-fold increase on day 3 and still 12-fold on day 7. Consistently, a more recent study carried out by Yilmaz and colleagues [141] demonstrated in patients that the numbers of DCs decreased transiently after stroke; furthermore, by analyzing human cerebral specimens with acute ischemic or hemorrhagic stroke, the authors found numerous DCs locating in the infarct area, supporting the hypothesis that the DCs in circulation were most likely to be their recruitment into the infarcted brain. On the other hand, it is also possible that the part of the DCs found in the lesion originates from local cerebral cells such as microglia [139].

Dual Roles of Innate Immune System Cells

Microglia

Activated microglia function analogously to circulating macrophages, with the ability to eliminate necrotic tissue and secrete proinflammatory cytokines including IL-1 β and TNF- α under ischemic condition, which exacerbate brain damage and promote leukocyte infiltration [134]; on the other hand, these cells also exert a neuroprotective potential by releasing anti-inflammatory cytokines like IL-10 and TGF- β to quell inflammation and benefit the outcome [142]. In the emerging concept, microglia are assorted into M1 and M2 phenotypes, like macrophages. The M1 phenotype is referred to as the classically activated phenotype and processes deleterious features by secreting proinflammatory cytokines and presenting antigen to T cells, whereas M2 microglia, the alternatively activated phenotype, are involved in the neuroprotection and tissue repair after ischemic injury [131]. Existing data suggests that overall suppression of microglia fails to benefit experimental outcome but, on the contrary, results in larger infarctions and doubling apoptotic neurons after ischemia [143], indicating the significance of microglia in alleviation and recovery of injury.

Macrophages

Traditionally, macrophages are viewed as a noxious component that amplifies ischemic injury and exacerbates secondary progression of ischemic lesions. Monocytes/ macrophages are recruited via CCL2/CCR2 axis, and deletion of CCR2 or CCL2 results in smaller cerebral infarcts, reduced monocytes/macrophages infiltration, and less proinflammatory mediator production, indicating a deleterious effect of these cells [144]. But the majority of studies demonstrate that macrophages in the injured region, regardless of the exact origin, are polarized into M1 and M2 phenotypes, and the M2 phenotype would show beneficial effects against ischemic damage [130]. Girard and colleagues [145] reported that macrophages that originate from peripheral monocytes might be cytotoxic, independently of their phenotype, while microglia may be protective. On the other hand, Hu and colleagues [146] demonstrated that the majority of microglia/macrophages within the infarct areas experience an M2-to-M1 shift during the stroke progress. Soon after the ischemia, macrophages of the M2 phenotype were present and exerted neuroprotective effects; while being at the later stage of injury, the M2 phenotype gradually transforms into the M1 phenotype and is involved in neuronal damage.

Neutrophils

Although elevated neutrophil accumulation is often observed during cerebral ischemia/reperfusion, the exact pathogenesis role of neutrophil infiltration is uncertain, and blocking the postischemia neutrophil recruitment is not necessarily leading to improved outcome [135]. In current concept, neutrophils confer to a functional heterogeneity and polarize into 2 distinct subsets, in which N1 phenotype mediates deleterious effect, while N2 phenotype exhibits neuroprotective effects [51].

Dendritic Cells

To date the exact role of DCs was not defined comprehensively, but most studies suggested that DCs increment was associated with worsened outcome [139]. In murine models, the numbers of DC in the brain correlated with the size of the brain lesion after pMCAO [140], whereas in patients with transient ischemic attack, acute ischemic stroke, and acute hemorrhagic stroke, the extent of the decrease of DCs significantly correlated with the clinical stage and the radiological size of stroke [141]. Moreover, suppression of DC migration and maturation by granulocyte-colony stimulating factor contributed to attenuation of cerebral inflammation and reduction of infarct size, exhibiting neuroprotective effects in murine models of tMCAO [147].

The mechanism of how DCs lead to poorer outcome remained elusive. Theoretically, DCs presented in the infarcted areas may activate T cells, induce a long-lasting immune response, and therefore lead to further neurological damage [141]. Additionally, the transient decrease of circulating DCs might lead to immunodepression, resulting in poststroke infections to worsen the clinical outcome in stroke patients [140].

Cytokines

Infiltration and activation of innate immune cells result in the production of various cytokines and inflammatory mediators, which either exacerbate or alleviate inflammatory damage to the ischemic brain tissue. Within the first 24 hours of cerebral ischemia in animal models, inflammatory cytokines interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor- α are upregulated dramatically by up to 40- to 60-fold and are believed to affect the infarct volume and tissue damage. Therefore, these cytokines stand among the most investigated inflammatory mediators [147]. IL-1 β is one of the neurotoxic cytokines released within 30 minutes after ischemic onset. Activated microglia appear to be the major source, whereas other immune cells may also express IL-1 β . Noxious effects of IL-1 β are well documented in numerous studies [148-149]. It is considered as a neurotoxic mediator that directly induces neuronal death and enhances the expression of cytokines. Furthermore, chronic release of IL-1 β is associated with increased expression of adhesion molecules and blood-brain barrier permeability, promoting further leukocyte infiltration [150]. In animal experiments, IL-1 α and IL-1 β double knockout significantly reduced infarct volume in cerebral ischemic mice models [151-152]. Additionally, meta-analysis of animal model studies also revealed that IL-1 receptor antagonist markedly reduced infarct volume by 38.2% [153].

Expressed within the first hour after ischemic onset, TNF- α is also an essential component involved in the early stage of cerebral ischemia [154-155]. Increased TNF- α level in serum was observed after stroke in patients, and the increase correlated infarct volume and severity of neurological impairment. TNF- α plays a dual role in brain injury. The neurotoxic effect of TNF- α might be attributed to direct induction of neuronal death and indirect promotion of leukocyte infiltration by elevating the expression of adhesion molecules and chemokine. However, in addition to the deleterious roles, TNF- α also exerts beneficial effects and mitigates inflammatory injury. TNF receptor knockout was reported to be associated with enlargement of infarct volume. Besides, TNF- α pretreatment would result in decreased infarct volume and reduced leukocyte infiltration after permanent middle cerebral artery occlusion in mice [156].

Compared with the former ones, reports on the role of IL-6 in experimental ischemic stroke are relatively fewer [157]. And current available evidence argues against a pathogenic role of IL-6 in ischemic stroke. On the contrary, initial studies indicated that IL-6 deficient has no impact on infarct volume in mice models [158]. Whereas studies later on argued that the failure of IL-6 deficient to affect infarct size might be due to hypothermia in the mice models, and with well-controlled hypothermia, IL-6 deficiency would lead to increased infarct volume and neuronal death, suggesting a neuroprotective role of IL-6 [157]. Furthermore, underlying mechanisms of the neuroprotective potential of IL-6 are partially revealed in recent studies, in which IL-6 was demonstrated to participate in angiogenesis [159], and Jung and colleagues [160] verified that IL-6 exerted this ability and protected ischemic tissue probably via STAT3 pathway. Moreover, in order to depict the interactions between various cytokines participating the postcerebral ischemic inflammation, Zeng and colleagues [161] adopted Bayesian network (BN) learning procedure to explore the underlying links among circulating cytokines and discovered that IL-6 modulated TNF- α and IL-1 β mRNA expression directly or indirectly, indicating that IL-6 is a key mediator of the inflammatory cytokine network during the postcerebral ischemic inflammation.

Attempts and Difficulties in Bench-to-Bedside Translation

A better knowledge of poststroke inflammatory response may give birth to novel therapeutic strategies against ischemic stroke. Thrombolysis with rt-PA is the only effective treatment to date. However, due to the time window of 4.5 hours and safety concerns, the portion of stroke patients that would benefit from this treatment is less than 5%. On the other hand, immunomodulatory therapies hold a great potential. Based on current knowledge, inflammatory response reacts immediately after ischemic onset while requiring hours to days to fulfill. Therefore, immunomodulatory treatment would have extended therapeutic window. In addition, immunomodulatory therapy would not increase the risk of hemorrhage. Finally, since the inflammation would be particularly exacerbated upon reperfusion, immunomodulation would ameliorate the potential reperfusion-induced exacerbation secondary to medical intervention and recanalization [120].

As is mentioned above, poststroke inflammation is featured by significant leukocyte infiltration, which is facilitated by the upregulated adhesion molecules. Based on this theory, clinical trials are conducted to explore whether the suppression of leukocyte infiltration by blocking ICAM-1 with monoclonal antibody enlimomab benefits clinical outcome in acute ischemic stroke patients. Disappointingly, the clinical trial ended up with negative results, suggesting an even worsened outcome upon enlimomab treatment [162]. In this study, 625 patients with ischemic stroke were enrolled, of whom 317 were randomized to receive enlimomab within 6 hours after stroke onset. Patients were not enrolled if they had received rt-PA. The treatment lasted over 5 days. However, when evaluated at day 90, patients that received enlimomab exhibited significantly worse Modified Rankin Scale score and higher mortality. Additionally, patients in enlimomab group experienced more adverse events, primarily infections and fever, than the placebo group. The negative effect may be interpreted by the murine source of enlimomab and the murine antibody might activate neutrophils through complement-dependent mechanisms and therefore amplify the inflammation and damage [114].

Likewise, UK-279, 276, a recombinant glycoprotein that selectively binds to the CD11b integrin to reduce neutrophil infiltration and infarct size in murine models, failed to exhibit any

benefit in patients. The study was a multicenter, doubleblind, randomized, placebocontrolled clinical trial to evaluate the efficacy of UK-279,276 in acute ischemic stroke. 966 patients were enrolled, among whom 887 had ischemic stroke and 204 were cotreated with rt-PA. Unfortunately, the trial was stopped early for futility in both subgroups receiving UK-279,276 no matter with concomitant rt-PA prescription or not [163].

In addition to the above-mentioned immune cells and cytokines that complicate the immune response to stroke, free-radicals also complicate the pathophysiological progress [164-165]. Therefore, free-radical trapping agents like NXY-059 are theoretically neuroprotective, and this hypothesis was confirmed in animal models. Furthermore, SAINT I study [166] found that NXY-059 significantly reduced disability rate in patients receiving this agent and markedly lowered hemorrhagic risk in those receiving rt-PA concomitantly.

Nonetheless, the subsequent SAINT II study [167] overturned both of these optimistic findings, stating that NXY-059 was ineffective for the treatment of acute ischemic stroke and had no effect on the hemorrhagic risk of rt-PA. Since SAINT II study presented larger sample size (3306 versus 1699), it is reasonable to consider the results from SAINT II study to be more reliable and the data from SAINT I may be false positive. However, even though no evidence was found of an interaction between rt-PA use and the effect of NXY-059 in either trial, we cannot completely rule out the possibility that the disparity between the two trials may derive from the higher frequency of rt-PA prescription use in SAINT II study (44% versus 29%) in which maximal improvement may be achieved already by rt-PA, in spite of NXY-059 [167].

There are several promising findings as well. In another trial, investigators adopted IL-1 receptor antagonist (IL-1Ra) in attempt to block cytokine cascade. This randomized phase II study [168] recruited 34 patients, among whom half were randomized to receive IL-1Ra and the others received placebos. None of the patients received rt-PA. Upon 3-month evaluation, patients that received IL-1Ra exhibited lower levels of inflammatory markers and better clinical outcomes. No adverse events were observed in both groups. This study indicated that IL-1Ra was safe and well tolerated among acute stroke patients and that IL-1Ra held a great potential to be a novel therapy, whereas the efficacy required further investigation.

The trial and errors in the attempt to find novel therapeutic strategies targeting poststroke inflammation have revealed several obstacles before successful clinical translation were put forward by Macrez and colleagues [114]. Firstly, it is still uncertain whether animal models of stroke can recapitulate human pathology and predict success in clinical trials needs. Secondly, safety, tolerance, and potential adverse events associated with therapeutic immunomodulation are of relevant concern. Finally, our knowledge of immune system and stroke is still limited. The interactions between stroke and immunity are elusive, and the role of inflammation in ischemic injury is complicated and sometimes conflicting. Therefore, safe and successful bench-to-bedside translation calls for a more comprehensive understanding of immune response after ischemic stroke before it could benefit stroke patients substantially.

THE ROLE OF ADAPTIVE IMMUNITY AFTER STROKE

The inflammatory processes detailed thus far occur in a short time window after infarction and rely on the innate immune system, which involves the rapid activation of low-

affinity receptors that recognize a wide range of targets. The immediate onset of this inflammatory cascade and the available experimental data on patterns of signaling during early immune activation do not support a substantial role in this process for the adaptive immune system, which relies on the clonal expansion of specific lymphocytes with high-affinity receptors to specific antigens. However, the general immune activation caused by cerebral ischemia raises the questions of whether the adaptive immune system is eventually activated and how it may contribute to the propagation and repair of brain injury after stroke. After stroke, the number of antigen-presenting cells in the brain increases, along with costimulatory molecules required for antigen presentation to lymphocytes. This antigen presentation results in the production of antibodies against brain antigens and T cells sensitized to brain antigens. Furthermore, successive mucosal administration of myelin antigens in experimental models results in the development of immune tolerance and protection from subsequent ischemic injury, suggesting that this immune response involves adaptive immunity and that modulating it may be protective. On the other hand, although lymphocyte-deficient mice are protected from ischemic brain damage, reconstituting them with T cells directed against non-CNS antigens worsens ischemic damage. In addition, mice lacking the necessary costimulatory molecules for antigen-specific T-cell responses are nevertheless vulnerable to ischemic damage. Therefore, it is unclear whether the release and presentation of CNS antigens during and after stroke result in an adaptive immune response directed against the CNS.

If such an autoimmune response was directed against the brain after stroke, its long-term implications would potentially be significant. Such immune activity would be expected to impair neuronal plasticity and functional recovery and contribute to the frequent incidence of post-stroke dementia. Such concerns are supported by the presence of inflammatory infiltrates in damaged areas of the brain years after stroke, as well as by persistently elevated titers of antibodies to brain antigens. Abnormal permeability of the blood-brain barrier has been linked to the radiographic white matter changes frequently associated with vascular disease and cognitive decline, and levels of inflammatory biomarkers such as C-reactive protein are associated with white matter changes, lacunar strokes, and loss of microstructural integrity as measured by diffusion-tensor imaging [169].

Therefore, it cannot be discounted that immune activation contributes to the alterations in this endothelial permeability and vascular dysfunction. On the other hand, immune cells such as microglia may be important for clearing deleterious cellular debris that can cause neurodegeneration. Further research will be required to determine what role, if any, immunity has in long-term outcomes after stroke, but elucidation of any potential mechanisms may open promising avenues for the development of new therapeutics to improve neurological recovery after brain injury.

RESOLUTION OF INFLAMMATION AND THE ROLE OF THE IMMUNE SYSTEM IN TISSUE REPAIR

The inflammation unleashed by cerebral infarction is followed by a carefully orchestrated process to clear necrotic debris and foster tissue repair. This reparative process releases mediators that actively bring the inflammatory process to a close. Phagocytosis of dead cells

by microglia and macrophages promotes the production of immunomodulatory cytokines, such as transforming growth factor β and interleukins. Although transforming growth factor β has numerous proinflammatory effects, in this context it helps to suppress inflammation by inhibiting helper T-cell responses and promoting regulatory T-cell development. Interleukin 10 has neuroprotective and anti-inflammatory properties, and its release helps to facilitate the resolution of inflammation and promotes the survival of remaining viable neurons [170].

In this evolving process, the same cells that were initially recruited in the inflammatory phase serve as important sources of growth factors required for neuronal sprouting, neurogenesis, angiogenesis, gliogenesis, and matrix reorganization see Figure 5. For example, microglia are required for the full expression of insulin like growth factor 1, which promotes neuronal sprouting after injury. Reactive astrocytes produce vascular endothelial growth factor, which is required for angiogenesis. Circulating CD34⁺ immune progenitor cells promote revascularization in infarcted brain tissue. This reparative aspect of immune cells raises expectations that they can be harnessed to augment neuronal repair and recovery after CNS injury. However, experimental efforts so far provide cautionary tales; for example, increasing vascular endothelial growth factor levels early after ischemia or in excessive amounts actually worsens injury. Such findings highlight the complexity of the immuneresponse to CNS injury and indicate that attempts to modify these interactions must be undertaken with care.

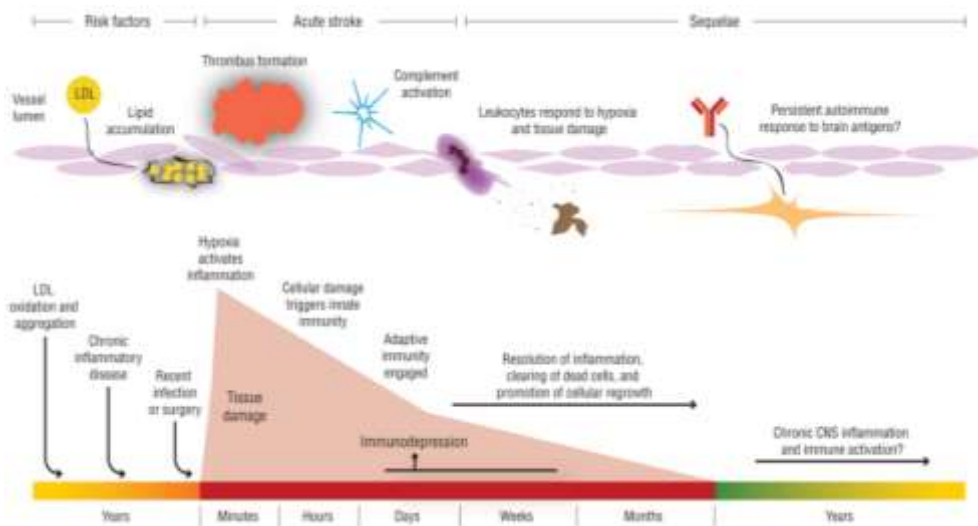


Figure 5. Progression of inflammation and immune activation in the development of stroke.

Chronic inflammation from atherosclerosis, autoimmune disease, and physiological stress results in progressive vascular injury that increases the risk of stroke. Acute occlusion of the cerebral vasculature produces intravascular hypoxia that triggers a rapid inflammatory response. As tissue damage proceeds, cellular components activate the innate immune response and set the stage for the engagement of adaptive immunity. Questions remain about whether this immune activation after stroke causes autoimmunity that affects neurological recovery. CNS indicates central nervous system; LDL, low-density lipoprotein.

BRAIN INJURY AND IMMUNOSUPPRESSION

Thus far, we have focused on the effects exerted by the immune system on the CNS after stroke. However, this interaction is bidirectional, and CNS injury has profound effects on immune function. Within days of stroke, patients develop significant immunodepression, marked by lymphopenia, upregulation of anti-inflammatory cytokines, and splenic atrophy [171]. This immunodepression clinically manifests in the high rate of systemic infections seen in the immediate poststroke period. Patients with stroke are especially at risk of pneumonia and urinary tract infections, and such infections may independently worsen neurological outcomes and increase mortality. Immunodepression may account for the inability of other factors (such as dysphagia) to fully account for the high rates of pneumonia seen in survivors of stroke.

Poststroke immunodepression seems to be mediated by catecholamines and steroids released by sympathetic activation after stroke. Cortisol and serum catecholamine levels correlate with susceptibility to infection after stroke, and experimental models have shown that steroid and adrenergic antagonists counteract lymphocyte apoptosis and reduce rates of infection after brain injury. Intriguing clinical observations associate β -blocker use with lower rates of pneumonia and mortality after stroke, but given the sparse nature of these data and the pleiotropic effects of β -blockers, further research will be required to determine the usefulness of such widely available drugs to modulate the immune response after stroke.

Other efforts to counteract poststroke immunodepression have involved the prophylactic administration of antibiotics after stroke to protect patients from common infections. Several randomized trials investigated whether this strategy improves outcomes after stroke, and a meta-analysis of their results indicates that antibiotic use reduced the rate of infections but not mortality [170]. However, these studies were underpowered to detect a meaningful difference in mortality rates, and further large trials will be required to answer this question. If antibiotic use is eventually shown to improve outcomes after stroke, questions will remain about the effects of such a strategy on microbial resistance patterns. Nevertheless, it is possible that a strategy of prudent poststroke antibiotic use may emerge as a cost-effective and safe strategy for improving outcomes in these vulnerable patients. In the meantime, physicians should be cognizant of the immunosuppressed state of their patients with stroke and should remain vigilant to expeditiously identify and appropriately treat infections in these patients.

RELATIONSHIP BETWEEN POSTSTROKE IMMUNODEPRESSION AND ADAPTIVE IMMUNITY

In speculating about why poststroke immunodepression occurs, on the surface it would seem to harm patients by increasing their risk of infectious complications. Although it may simply be a maladaptive response that stems from inherent aspects of the design of the CNS and immunesystem, immunodepression may serve to protect the CNS from the development of adaptive immune responses directed against self. Recent data indicate that the CNS undergoes regular immune surveillance by circulating lymphocytes. Central nervous system components are not routinely presented to these lymphocytes in such a way as to sensitize them and launch an immune response against the CNS. However, in the absence of

countervailing factors, such antigen presentation would be expected to occur after CNS injury and compromise of the blood-brain barrier. Therefore, the immunodepression seen after stroke may serve a beneficial purpose in limiting the development of such autoimmunity. Such considerations suggest that a detailed understanding of the many facets of the interactions between the CNS and the immune system is needed to guide any interventions to modify these interactions and improve outcomes.

IMMUNE RESPONSE TO ACUTE STROKE

Traditionally, several obstacles to a full immune response have been thought to exist in the CNS. These obstacles include the blood-brain barrier (BBB), the lack of cerebral lymphatic vessels, the apparent inefficiency of microglia and astrocytes for antigen presentation to T cells, the high rate of apoptosis in cells that cross the BBB, and the influence of contact-dependent or contact-independent mechanisms between neural cells that downregulate the crosstalk of these cells with lymphocytes and other immune cells [171-175]. Recent data, however, have dramatically altered this viewpoint by revealing that the CNS actively interacts with the immune system, both directly and via intermediates [176-177].

Mounting evidence indicates that acute stroke is followed by a complex interplay between the CNS and the immune system. The concentration of various cytokines is increased in the cerebrospinal fluid and blood of patients with acute stroke, and these changes are associated with clinical events including infection, functional outcome and mortality [178-180]. In this section, we will discuss the recent clinical and experimental studies on the innate and adaptive immune responses following acute stroke, including cytotoxic and cytoprotective effects, and the role of stroke-induced immunodepression in increasing the risk of infection. It is suggested that a better understanding of these findings could promote the identification of novel therapeutic targets for this devastating condition.

Innate Immune Response after Stroke

Phylogenetically, innate immunity is the oldest system of host defence and provides the early response against microbes and products of injured cells. It consists of physical and chemical barriers, cellular components such as macrophages, neutrophils and natural killer cells, blood proteins such as members of the complement system and mediators of inflammation, and cytokines, which are important regulators of the immune response (Figure 5).

Damage-Associated Molecular Patterns

Acute stroke stimulates an inflammatory cascade in the occluded vessel, the arterial wall and the brain parenchyma.¹¹ Destruction of neural cells leads to release of damage-associated molecular patterns (DAMPs) to the extracellular environment, which activate the innate and adaptive arms of the immune system and further stimulate the inflammatory cascade [181-

182]. Neurons are particularly vulnerable to ischaemia, and rapidly release DAMPs that attract and activate neighbouring microglia. DAMPs include the chromatin-associated protein termed high mobility group protein B1 (HMGB1), uric acid, heat shock proteins, ATP, S100 proteins, heparan sulphate, DNA, and RNA [183-184].

The release of DAMPs can be associated with either beneficial or harmful effects: inhibition of HMGB1 was neuroprotective in several experimental studies, whereas application of HMGB1 promoted functional recovery. Administration of uric acid was shown to be neuroprotective after experimental transient focal brain ischaemia, and higher endogenous levels of uric acid were associated with more-effective reperfusion therapy in patients with acute stroke. By contrast, reduction of the level of uric acid was associated with attenuated neutrophil recruitment to the liver after acetaminophen-induced liver injury. A possible explanation for the organ-specific effects of uric acid in these studies is that the brain has a considerably lower endogenous antioxidant capacity than does the liver, which could cause the antioxidant capacity of uric acid to prevail over its proinflammatory effects in the brain but not in the liver [185-187].

Innate Immune Receptors

Innate immune cells sense exogenous pathogens and endogenous danger signals through pattern recognition receptors that include Toll-like receptors (TLRs), RIG-1-like receptors, NOD-like receptors, C-type lectin receptors and AIM2-like receptors [188]. These receptors activate downstream signalling pathways, such as nuclear factor- κ B, mitogen-activated protein kinase and type 1 interferon pathways, which in turn upregulate proinflammatory cytokines, chemokines, reactive oxygen species, and costimulatory signals. Activation of these receptors also promotes priming, activation and clonal expansion of antigen-specific T cells. The importance of these receptors in mediating tissue damage in stroke was highlighted in studies that showed smaller infarcts and less-severe brain haemorrhages in transgenic mice lacking TLR2 or TLR4 than in wild-type mice. Moreover, a better outcome was observed in patients with stroke who exhibited reduced expression of TLR4 in monocytes [189-192].

Monocytes

Monocytes are multifunctional innate immune cells with crucial roles in the regulation of inflammation and tissue repair. In rats, monocytes were found in the damaged brain tissue shortly after acute stroke. In patients with acute stroke, these cells increased in number in the blood, and showed phenotypic changes comprising reduced expression of antigen-presenting molecules and low production of proinflammatory tumour necrosis factor, although production of anti-inflammatory IL-10 remained unchanged. The magnitude of these changes was associated with the risk of poststroke infection, whereas the balance between classic CD14⁺ proinflammatory monocytes and minor populations of reparative CD16⁺ monocytes was associated with stroke outcome. These monocyte subtypes could represent populations of cells that differentiate into M1 and M2 macrophages, respectively. M1 macrophages promote strong T-helper-1 (TH1) responses, whereas M2 macrophages support TH2 responses and might play a part in resolution of inflammation [193-197].

The Complement System

The complement system is part of the innate immune response, and its activation has been described in clinical and experimental stroke. The lectin pathway of complement activation is initiated by mannose-binding lectin (MBL) and MBL-associated serine proteases. Patients with stroke who have an MBL low genotype express lower levels of the complement components C3, C4, and C-reactive protein, and have better functional outcomes than patients with an MBL-sufficient genotype. Similarly, following brain ischaemia, transgenic mice lacking MBL show reduced infarct size, C3 deposition and neutrophil infiltration, and better outcomes than do wild-type mice. Together, these findings emphasize the clinical relevance of this component of the innate immune response after stroke [198-200]

Adaptive Immune Response After Stroke

Adaptive immunity is an evolutionary advancement that provides more-effective defence mechanisms against microbes and injured cells than does innate immunity. The adaptive response is slower than the innate response but has exquisite specificity for a large number of microbial and nonmicrobial substances. This arm of the immune system also has the capacity to 'remember' previous exposure to a given pathogen and mount a more rapid response on repeat infection. The main cellular components of this response are B and T lymphocytes, secreted products of these cells such as antibodies and cytokines, antigen-presenting cells, and effector cells. The adaptive response is initiated by recognition of antigens by lymphocytes, which respond by proliferating and differentiating into effector cells that can have cytotoxic or cytoprotective effects (Figure 5).

Cytotoxic Effects

Effector T Cells

Recent experimental studies suggest that different T-lymphocyte subpopulations, but not B cells or natural killer cells, are involved in the evolution of brain infarction and accompanying neurological deficits. During the early phase of stroke, T cells cannot cross the BBB, and probably exert their detrimental effects independently of antigen recognition.

The invasion of lymphocytes into the brain parenchyma is regulated by interaction of the leukocyte-expressed very late antigen with endothelial vascular adhesion molecule 1. The extravasation of lymphocytes to the brain parenchyma is facilitated by oxidative stress, by proteases expressed in vascular cells and released by leukocytes, and by other inflammatory mediators that contribute to alteration of BBB permeability. Infiltrating T cells are the main source of IFN- γ , which mediates delayed neurotoxic effects in the ischaemic brain tissue [201-202].

$\gamma\delta$ T cells and the cytokines IL-23 and IL-17 seem to have a crucial role in maturation of brain infarction. Activation of $\gamma\delta$ T cells does not require stimulation of the antigen-specific T-cell receptor, and is probably induced by IL-23 secreted by infiltrating macrophages, and by activation of surface TLRs, such as TLR2 via DAMPs (discussed above). In a mouse model of stroke, activated $\gamma\delta$ T cells infiltrated the brain and in turn produced the

The diagram illustrates the pathogenesis of stroke and the role of the immune system. It is divided into three main regions: Cerebrospinal fluid (top), Brain tissue (middle), and Blood vessel (bottom). Stroke is indicated by lightning bolts on the left. In the Cerebrospinal fluid, Cerebrospinal fluid drainage is shown. In the Brain tissue, Neuronal death leads to Antigen release and DAMPs, which activate microglia. Resting microglia also become activated. Oxidative stress (O_2^-) leads to Inflammation. In the Blood vessel, BBB damage leads to Complement activation (C5b-2) and Blood clotting. Leukocyte infiltration occurs from the blood into the brain tissue. The diagram also shows the activation of T cells (Activated T cell) and the role of the Cervical lymph node (Activated B cell, Activated T cell, Soluble antigen, Antigen recognition). The final outcome is Tolerance or damage, leading to Lymph and Blood flow.

Brain ischaemia triggers a complex cascade of events that include oxidative stress, microglial and complement activation, blood clotting, vascular damage, cellular infiltration of the ischaemic tissue, and activation of the innate and adaptive immune systems. DAMPs released by necrotic cells are sensed by pattern recognition receptors such as Toll-like receptors, which are expressed by immune cells and neural cells. The adaptive immune system is also stimulated by damaged brain cells through the release of brain antigens that drain to peripheral lymph nodes and encounter antigen-presenting cells, and T and B cells. This encounter can cause a T-cell-mediated reaction in the brain, and can lead to generation of antibodies against specific brain-derived molecules. Whether these effects cause autoimmunity or facilitate induction of tolerance has not been fully elucidated. Abbreviations: BBB, blood–brain barrier; DAMPs, damage-associated molecular patterns; T^{REG}, regulatory T.

Invasion of lymphocytes after acute stroke can expose brain epitopes that are normally ‘hidden’ from the immune system, and can promote priming and activation of lymphocytes that are reactive to CNS antigens. Lymphocytes that are specific for CNS antigens could boost inflammatory responses in the damaged brain tissue, thereby contributing to a worse

stroke outcome. Some patients with stroke show higher serum antibody titres and numbers of circulating T cells specific for CNS antigens than do healthy controls, suggesting that the inflamed CNS tissue induces self-directed immune responses [210-211]. The emergence of autoreactive immunity after stroke was also suggested by recent studies that found an increased presence of neuronal and myelin antigens in the cervical lymph nodes and palatine tonsils of patients with acute stroke. The antigens were found in macrophages located near activated T cells, and were associated with improved or impaired stroke outcome, depending on the relative predominance of neuronal or myelin epitopes, respectively [212]. The reasons for the discrepant effects of neuronal or myelin epitopes have not been elucidated and deserve further study. Other studies showed that immunization with myelin antigens worsened outcome in experimental models of cerebral ischaemia, and that systemic inflammation at stroke outset, or early in the disease course, primed autoreactive immune responses to CNS antigens such as myelin basic protein (MBP) and glial fibrillary acidic protein [213-215].

Collectively, these studies highlight the occurrence of self-reactive responses after acute brain ischaemia, although they do not clarify whether antigen-specific activation of immune cells is beneficial or detrimental, or how these effects are influenced by infections that precede or follow acute stroke. The mechanisms that govern protective versus non-protective T-cell responses specific to CNS antigens after stroke could depend on the anatomy of brain damage (that is, grey matter versus white matter damage), effector T-cell phenotype, activation of regulatory T (TREG) cells, genetic background, and/or other factors that determine the strength and timing of autoreactive T-cell activation.

A better understanding of the mechanisms underlying autoreactivity after acute stroke could inform the development of new therapeutic strategies in the near future. Recombinant T-cell-receptor ligands comprising partial major histocompatibility complex class II (MHC-II) molecules have been developed that may act as partial T-cell-receptor agonists and direct autoreactive T cells to become nonpathogenic. For example, RTL551—which contains the myelin oligodendrocyte glycoprotein (MOG)-35-55 antigenic peptide—was successfully used as a neuroantigen-specific immunomodulatory treatment in a model of cerebral ischaemia, suggesting therapeutic potential in human stroke [216].

Cytoprotective Effects

T cells that are specific to myelin antigens can reduce secondary neurodegeneration, enhance neurogenesis and promote recovery after CNS injury, indicating that autoimmune responses might be protective under experimental conditions. Lymphocytes that had been tolerized to specific CNS antigens provided long-lasting local immunosuppression, leading to immunological tolerance on restimulation. For example, induction of immunological tolerance by intranasal or oral application of MBP or MOG weeks before stroke onset reduced infarct size and improved recovery in rodent stroke models. Intranasal administration of MBP also increased the frequency of Treg cells, and reduced the extent of TH1 responses to MBP. In addition, nasal instillation of E-selectin, which is specifically expressed on activated endothelium, was cytoprotective and prevented ischaemic and haemorrhagic strokes in rats. Overall, these findings could indicate that in patients with a history of stroke, antigen-specific immunomodulation might prevent recurrent stroke and improve stroke outcome, although recent studies have raised concerns about the potential to induce deleterious autoimmunity through mucosal administration of CNS antigens [217-223].

Regulatory T Cells

Numerous studies in animal models have shed light on the role of endogenous Treg cells in cerebral ischaemia. These cells are characterized by expression of the transcription factor forkhead box protein P3 (FOXP3) and are important regulators of immune homeostasis, both in health and immune-mediated diseases [224]. Treg cells can exert their anti-inflammatory effects by a direct interaction with various other cells, as well as by secretion of the cytokines IL-10 and transforming growth factor- β (TGF- β).

Interestingly, after extensive brain ischaemia, Treg cells are less susceptible to undergoing apoptotic cell death than are other splenocytes, suggesting a brain damage-related shift towards an anti-inflammatory phenotype in the adaptive immune system [225]. Indeed, endogenous Treg cells have an important protective role in ischaemia, as depletion of these cells in mice considerably increased infarct size and behavioural deficits after brain ischaemia.⁵⁷ These effects did not become evident until 3–7 days after middle cerebral artery (MCA) occlusion. Moreover, depletion of Treg cells exacerbated damage in models of moderate cortical or subcortical ischaemia, but not in models of extensive ischaemia—a finding that has been reproduced using another paradigm [226]. In contrast to the late migration of Treg cells into the CNS and the delayed effect of these cells on tissue damage, Treg-cell depletion has profound effects on early activation of and cytokine production by brain microglia. Moreover, depletion of Treg cells enhances activation of invading proinflammatory T cells, eliciting increased expression of the inflammatory cytokine IFN- γ . The protective effect of Treg cells in animal models is mediated by IL-10, which is consistent with the beneficial effects of therapeutic IL-10 delivery to the brain [227].

B Cells

Regulatory B cells were recently reported to have beneficial effects on the ischaemic brain as early as 24–48 h after MCA occlusion.⁽⁸⁷⁾ Lack of B cells substantially increased infiltration of various leukocyte subpopulations into the brain, and reduced their functional activation. Conversely, transfer of B cells into the brain reduced infarct size and production of inflammatory cytokines by peripheral T cells [228].

An Integrated Immune Response

Brain ischaemia results in oxidative stress, complement activation, and blood clotting (Figure 5). These mechanisms contribute to vascular damage and lead to infiltration of the brain parenchyma by immune cells according to a temporal pattern that is coordinated by upregulation of chemoattractant and adhesion molecules. Neutrophils release proteases that can further damage BBB integrity and exacerbate oxidative stress [229]. The early clinical course of stroke is influenced by circulating monocytes and macrophages—cells that also participate in clearance of debris and damaged cells at later stages, which represents an important step before regenerative processes can be initiated. Dendritic cells and lymphocytes accumulate in the ischaemic brain parenchyma early after stroke onset, although their contribution to immune regulation in post-ischaemic inflammation remains to be fully elucidated. Glial cells develop an inflammatory phenotype in response to ischaemia, and release mediators that attract neutrophils, monocytes and lymphocytes. DAMPs that are

released by necrotic cells contribute to amplification of the innate immune response and activation of T cells [230].

The adaptive immune system is activated by acute stroke, as cell death results in release of brain antigens to the extracellular milieu. These antigens can subsequently reach the cervical lymph nodes and palatine tonsils by passing through the cribriform plate and travelling along basement membranes in the walls of capillaries and cerebral arteries. Some reactive forms of microglia, infiltrating macrophages and dendritic cells express MHC-II receptors and can, therefore, present brain antigens to T cells and B cells in lymphoid tissue. These cells can also provide the cytokine stimulus for T-cell activation [230].

In animal models, the presence of brain antigens in the cervical lymph nodes is sufficient to stimulate a T-cell-mediated reaction [231]. Antigen-specific T lymphocytes probably circulate from nodes in other parts of the body to cervical lymph nodes, where they receive an 'address' to target the brain through the expression of integrins on their surface, thereby enabling them to specifically target the injured cerebral hemispheres. B-cell activation leads to generation of antibodies against specific brain-derived molecules, whereas T-cell activation might be involved in autoimmune or tolerance reactions. The interplay of various cytokines influences adaptive responses. Anti-inflammatory molecules, such as IL-10 and TGF- β , and regulatory cells, such as T_{reg} and B-regulatory cells, can have a prominent beneficial role, probably by dampening proinflammatory and self-directed reactions to the brain tissue. Serum IL-10 levels have been suggested as a marker of stroke severity and outcome in patients. However, the effects of therapeutic IL-10 delivery have not yet been examined in patients [232].

Stroke-Induced Immunodepression

Acute stroke can cause a stroke-induced immunodepression (SIID) syndrome. The proportion of patients with stroke who develop SIID is unknown, as a commonly accepted definition of SIID and representative investigations in large stroke populations are lacking. The rate of SIID can, however, be estimated as 30% according to the frequency of poststroke infections. Manifestations of systemic immunodepression are not unique to stroke or to other acute injuries to the CNS, as they can also occur after traumatic injury, severe burns or brain surgery. These conditions all seem to be accompanied by a large outflow from the autonomic nervous system that profoundly affects the innate and adaptive immune response and is responsible, at least in part, for the increased susceptibility to subsequent bacterial infections (Figure 7) [233-234].

CNS injury can also directly induce immunodepression. This unique feature of acute brain damage might cause a different systemic immune response to that induced by severe body trauma without CNS injury, and is a matter of intense research [233]. The role of hepatic invariant natural killer T (iNKT) cells in mice and the release of acetylcholine by splenic memory T cells in SIID (Figure 6) were recently highlighted. iNKT cells have a highly restricted repertoire of T-cell receptors that recognize lipid antigens presented by CD1. After experimental stroke in mice, iNKT cells showed a noradrenergic-mediated anti-inflammatory phenotype that increased susceptibility to infections. The anti-inflammatory

phenotype was reversed with administration of the β -blocker propranolol or the immunomodulator α -galactosylceramide [235-237].

Teleologically, SIID could represent an adaptive response to limit ischaemia-induced inflammation in the brain, although it can facilitate the emergence of infections owing to reduced inflammatory drive [237]. Lymphocytopenia is a consistent hallmark of SIID, but conflicting evidence exists regarding the functional status of the surviving T lymphocytes. Specifically, previous studies found that proinflammatory T cells from patients with SIID responded less strongly to *in vitro* stimulation than did such cells from healthy controls [238], but recent studies have shown that both effector and Treg cell subpopulations sustain their proliferative capacity after stroke in mouse models and in humans [239-240].

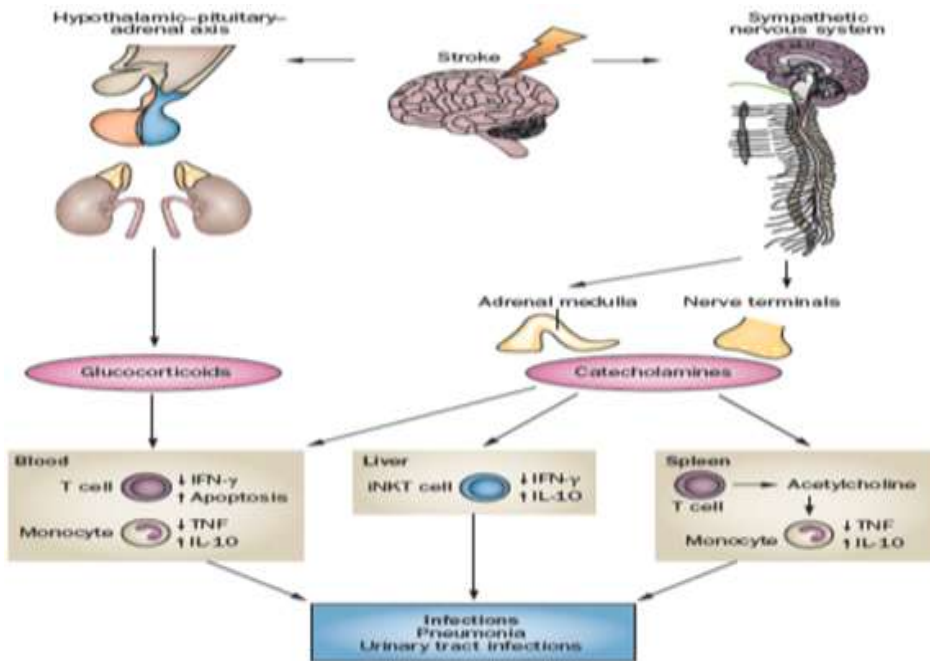


Figure 7. The anti-inflammatory reflex and poststroke infection.

The CNS modulates the activity of the immune system through complex humoral and neural pathways that include the hypothalamic–pituitary–adrenal axis, the vagus nerve and the sympathetic nervous system. The hypothalamus is functionally linked with autonomic centres, allowing the synchronization of neuroendocrine (glucocorticoid) responses with the cholinergic pathway, which together suppress the peripheral release of inflammatory cytokines from T cells, monocytes and macrophages, and promote the release of anti-inflammatory cytokines such as IL-10. Similarly, release of noradrenaline from a dense network of neurons throughout the brain and from peripheral organs, including the adrenal medulla, the liver and the spleen, induces a pronounced anti-inflammatory phenotype in lymphocytes, monocytes and macrophages. Together, these mechanisms limit the inflammatory response in the brain but can facilitate the emergence of infections such as pneumonia and urinary tract infections. Release of catecholamines from nerve terminals may induce profound behavioural changes in hepatic iNKT cells, and release of acetylcholine by splenic memory T cells, both of which could prevent inflammation and promote the emergence of infection after stroke. Abbreviations: iNKT, invariant natural killer T; TNF, tumour necrosis factor.

A crucial component of SIID in patients with stroke and in experimental conditions [241-242] is overactivation of the adrenergic system, which acts on peripheral immune cells to cause a switch from a proinflammatory TH1 response to an anti-inflammatory TH2 response. Sympathetic activation can also result in gastrointestinal dysmotility [243], which increases the risk of developing aspiration pneumonia.

Neuroprotection by Antibiotics

Antibiotics can be used after acute stroke to prevent infection, but could also offer neuroprotection. Minocycline does not cover the full spectrum of bacteria that commonly cause pneumonia and urinary tract infections, but is used to treat patients with acute stroke owing to its neuroprotective properties. Minocycline has anti-inflammatory effects and improves outcome in experimental stroke studies, although these effects were not reproduced in a recent preclinical drug evaluation [244-248].

β -Lactams are another class of antibiotics with reported neuroprotective properties. In this case, neuroprotection is mediated by increased glutamate transporter expression. In a neonatal rat model of cerebral ischaemia, pretreatment with ceftriaxone significantly reduced brain injury and hippocampal cell apoptosis, restored myelination in the external capsule, and increased expression of the glutamate transporter GLT1 in cortical neurons [244]. GLT1 is mostly expressed in mature astrocytes and is the main glutamate transporter in the brain. Excessive glutamate release induced by cerebral ischaemia can cause neuronal damage, and increased glutamate transporter activity could, therefore, explain the putative neuroprotective effects of ceftriaxone. Ceftriaxone has a broad spectrum of activity against bacteria that cause infection after acute stroke. The combined properties of ceftriaxone, therefore, suggest that this drug could prevent infection after acute stroke as well as limiting neuronal damage, although its effects on functional outcome in patients with stroke remain unknown [244].

ROLE OF T CELLS IN ISCHEMIC STROKE

Following an ischemic stroke, T lymphocytes become activated, infiltrate the brain, and appear to release cytokines and reactive oxygen species to contribute to early inflammation and brain injury. However, some subsets of T lymphocytes may be beneficial even in the early stages after a stroke, and recent evidence suggests that T lymphocytes can also contribute to the repair and regeneration of the brain at later stages. In the hours to days after stroke, T-lymphocyte numbers are then reduced in the blood and in secondary lymphoid organs as part of a 'stroke-induced immunodeficiency syndrome,' which is mediated by hyperactivity of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, resulting in increased risk of infectious complications. Whether or not poststroke T-lymphocyte activation occurs via an antigenin dependent process, as opposed to a classical antigen-dependent process, is still controversial. Although considerable recent progress has been made, a better understanding of the roles of the different T-lymphocyte subpopulations and their temporal profile of damage versus repair will help to clarify whether T-lymphocyte targeting may be a viable poststroke therapy for clinical use.

T lymphocytes are central to the development of a sustained inflammatory response and there is now good evidence that these cells accumulate in the postischemic brain within a few hours of reperfusion [249]. Here in this section of the chapter we will briefly summarize the emerging evidence of complex roles of T cells in brain injury after stroke. As shall be discussed, T cells are sources of pro-inflammatory cytokines and cytotoxic substances, such as reactive oxygen species, in the brain after stroke, which likely contribute to neuronal death and poor outcomes. However, future therapeutic efforts to treat stroke by targeting T cells must take into account the crucial roles that T cells have in host defense against invading pathogens, which appears to be critically important in the period following a stroke. Furthermore, recent evidence for a novel role of T cells in promoting brain tissue repair and regeneration in the weeks and months after stroke will also be discussed in this section.

T-Lymphocyte Subtypes and Characteristics

T lymphocytes are bone marrow-derived leukocytes that mature in the thymus and have an integral role in the adaptive immune system (i.e., the division of the immune system that recognizes specific pathogens and can generate immune memory). As such, they are vital for clearing pathogens that cannot be cleared by the innate immune system alone (i.e., the division of the immune system that initially defends the body from infection, in a nonspecific manner) [250]. Several T-cell subtypes exist, and these can be differentiated by their unique expression profiles of specific coreceptor proteins. CD3 is expressed on the surface of 95% of the T cells that have exited the thymus and which are immunocompetent. The CD3⁺ subset comprises both CD4⁺ (or T-helper cells; TH) and CD8⁺ (or cytotoxic T cells; TC) cells in approximately equal proportions [250] (Figure 8).

TH cells do not kill cells directly, but instead help to activate other immune cells including TC cells, and can be further differentiated into three major types defined by the cytokines they secrete. Thus, TH cells can promote either: cell-mediated or inflammatory immunity (i.e., TH1 cells); humoral and allergic responses (i.e., TH2 cells) [251-252]; or inflammatory immunity to clear pathogens distinct from those handled by TH1 or TH2 cells, including fungi (i.e., TH17 cells) [253]. On activation, TH1 cells secrete proinflammatory cytokines such as IFN- γ (interferon- γ), TNF (tumor necrosis factor), and LT- α (lymphotoxin), whereas TH2 cells produce antiinflammatory cytokines such as interleukin-4 (IL-4) and IL-10. TH2 cells are thought to suppress pro-inflammatory immune responses, and commonly appear later in an immune response [251]. TH17 cells secrete IL-17, IL-21, and IL-22 [252]. An important CD4⁺ T-cell subtype that accounts for 10% of all CD4⁺ T cells [254] and which expresses CD25 and the transcription factor Foxp3, is the regulatory T cell (Treg; i.e., CD4⁺ CD25⁺Foxp3⁺ cells). Treg cells act to limit the immune response and thus prevent autoimmune disorders [255] via their release of transforming growth factor- β and IL-10 [202].

As their name suggests, the cytotoxic TC cells can directly kill cells that contain intracellular pathogens, such as viruses, through the release of the cytotoxins, perforin, and various granzymes (resulting in necrosis), or by the Fas-FasL pathway (resulting in apoptosis) [256]. TC cells also produce pro-inflammatory cytokines, such as IFN- γ and TNF, which serve to block viral replication as well as to promote the activation of other elements of the immune system [257]. Finally, 'unconventional' T lymphocytes that act to link the innate and

adaptive immune systems (and are often called innate-like lymphocytes) include gd T cells (B5% of T cells; the only T cells that do not express CD3) and natural killer T (NKT) cells [258].

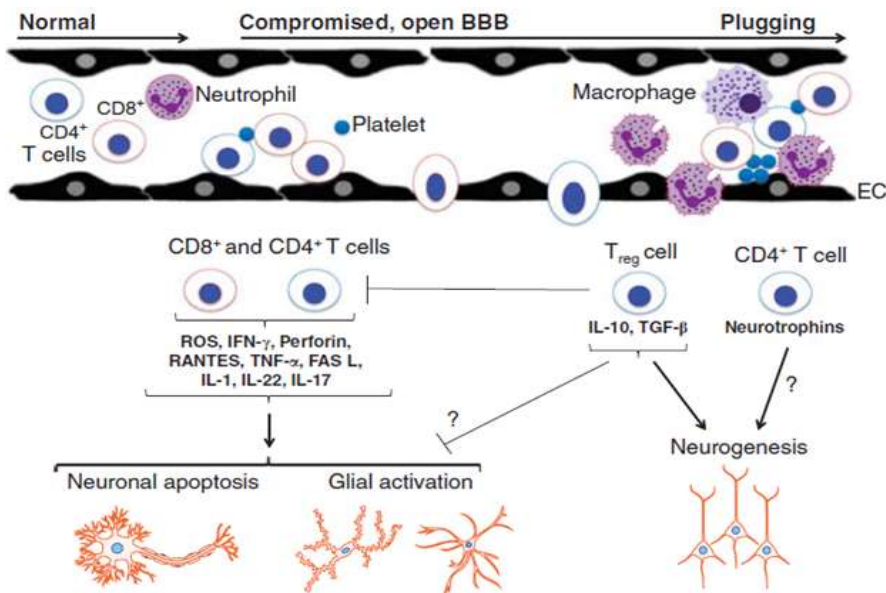


Figure 8. Potential actions of T lymphocytes in the brain following stroke.

Following cerebral ischemia and reperfusion, circulating T lymphocytes interact with neutrophils, macrophages, platelets, and endothelial cells (ECs), and may cross the blood–brain barrier (BBB) to infiltrate the injured tissue. The two broad subpopulations of mature circulating T lymphocytes are T-helper cells (i.e., CD4⁺) and cytotoxic T cells (i.e., CD8⁺). It appears that both subpopulations contribute significantly to acute poststroke brain injury via several mechanisms. The small subpopulation of CD4⁺ regulatory T cells (i.e., Tregs) may act to limit some of these damaging effects by other T cells. In addition, CD4⁺ T cells, perhaps especially Tregs, may contribute to later repair processes, such as neurogenesis, during brain recovery (adapted from VH Brait et al., 2012 npg).

Recent studies of the postischemic brain microcirculation have revealed that the accumulation of T cells in postcapillary venules is generally accompanied by the recruitment of adherent platelets, suggesting that the venules assume both a proinflammatory and prothrombogenic phenotype after ischemic stroke [259]. The net impact of the accumulated platelets in the postischemic microcirculation remains unclear; however, inhibition of platelet tethering and adhesion to vascular endothelial cells (the early phases of platelet activation) has produced reductions in cerebral infarct volume [17, 260–261]. Furthermore, inhibition of early platelet adhesion and activation maintains cerebral blood flow during reperfusion [262]. A novel concept of ‘thrombo-inflammation’ suggests that there is a strong link between pathways of thrombus formation and inflammation, and recent evidence suggests that specific early platelet adhesion/activation mechanisms may in fact link these pathways to exacerbate infarct development following cerebral I–R [263]. However, it is currently unclear as to whether specific interactions between T lymphocytes and platelets have an important role in stroke outcome, as T-cell deficiency is reported to have no effect on thrombus formation [264].

POTENTIAL MEDIATORS OF DAMAGE BY T LYMPHOCYTES AFTER STROKE

Cytokines, Chemokines, and Cytotoxins

The mechanisms of T-cell-mediated brain injury following stroke are currently unclear. Classically, T cells kill bacteria- and virus-infected cells either via the release of cytokines (in the case of TH and TC cells) or cytotoxins (also by TC cells) [250], and so it is plausible that similar actions may exacerbate poststroke brain inflammation. Cytokines and chemokines released by TH and TC cells are likely to increase expression of vascular adhesion molecules and attract other immune cells into the brain, resulting in widespread apoptosis [265]. Alternatively, TC cells may directly cause either cell necrosis or apoptosis via the release of cytotoxins or the activation of the Fas receptor [256]. Neutralization of certain T-cell-derived cytokines (e.g., IL-17, IL-12, IL-23) was found to reduce infarct volume and improve neurologic outcome scores over 7 days poststroke [51, 206], consistent with these T-cell-derived cytokines contributing to brain injury early after I-R. Furthermore, Liesz et al., (2009b) showed that CD3⁺ T cells are a major source of the damaging pro-inflammatory cytokine, IFN- γ , in the ischemic hemisphere. When neutralizing antibodies against IFN- γ were administered into the cerebral ventricles, there was a significant reduction in infarct volume [203]. Consistent with these results was the finding that reduced mRNA expression of various proinflammatory cytokines, chemokines, and chemokine receptors (TNF- α , IL-6, IL-10, IP-10, CCR1, CCR2, CCR3, and CCR5) occurs after stroke in the brains of SCID mice compared with wild-type mice [49]. Taken together, current evidence suggests that T cells (potentially excluding the immunomodulatory subset of Treg cells) represent a major source or stimulus of pro-inflammatory cytokines in the brain following stroke. Moreover, stroke in perforin-deficient mice produce a significantly smaller infarct volume, suggesting that the cytotoxin perforin, released by TC cells, contributes to ischemic damage [209].

Reactive Oxygen Species

There is recent evidence that T lymphocytes may contribute to oxidative tissue injury following stroke, potentially via the release of NADPH oxidase type 2 (Nox2)-derived superoxide. First, it is quite clear that oxidative stress due to excessive levels of reactive oxygen species is a major mechanism of poststroke brain injury. Studies of stroke in mice either overexpressing antioxidants or deficient in pro-oxidant enzymes have reported smaller infarct volumes than in wild-type controls [266-268], and conversely, studies of antioxidant-deficient mice have found larger infarcts [269-270]. Moreover, several studies in Nox2-deficient mice have reported substantially smaller infarct and edema volumes, and less blood-brain barrier disruption than wild-type controls, pointing to Nox2 oxidase as a key source of damaging superoxide in the brain after stroke [271-273]. T lymphocytes are now known to contain a functional Nox2 oxidase and, at 24 hours following stroke, circulating T cells produce B7-fold greater amounts of Nox2-derived superoxide than do T cells from control mice. Interestingly, much greater levels of superoxide are generated by circulating T cells compared with spleen-derived T cells after stroke [249, 274], consistent with the possibility

that exposure to the circulation (and/or postischemic brain tissue) upregulates Nox2 oxidase activity in T lymphocytes. In addition to Nox2, a recent study by Kleinschnitz et al., [50] has identified NADPH oxidase type 4 (Nox4) as an important source of oxidative stress and an effective therapeutic target in acute stroke. Nox4 was induced in human and mouse brain following ischemic stroke, and mice deficient in Nox4 (Nox4^{-/-}) developed smaller infarct volumes, had improved functional outcomes, and were largely protected from oxidative stress, blood–brain barrier leakage, and neuronal apoptosis, after both I–R and permanent cerebral ischemia.

Mechanism(s) of T-Lymphocyte Activation after Stroke

There is currently a major unanswered and controversial question regarding the mechanism(s) of T-lymphocyte activation following stroke. Based on the information gained from decades of study in the area of immunology, it is apparent that the activation and infiltration of T cells into the brain following stroke is too quick for it to occur via a classical antigen-dependent response (Offner et al, 2006a). Traditionally, it has been believed that for T cells to infiltrate into tissues, cellular activation leading to a change in the expression of surface molecules (e.g., VLA-4, CCR5, and CD44) is required [250,275]. Classical antigen-dependent activation of naive T cells comprises two main steps and takes 7 to 10 days [276]. The first step involves the binding of the T-cell receptor (TCR) to the antigen presented on the major histocompatibility complex on the surface of an APC (antigen-presenting cell). The second step involves the binding of costimulatory molecules on the T cell and the APC, such as CD28 on the T cell and CD80 (B7.1) and CD86 (B7.2) on the APC [277]. An elegant recent study by Kleinschnitz et al., in 2010 indeed found evidence that the antigen-dependent activation of T cells is not required for them to contribute substantially to the infarct volume present at 24 hours after cerebral I–R. Through the use of various transgenic mice, including Rag1^{-/-} mice reconstituted with CD3⁺ T cells, TCR-transgenic mice (bearing a single CD4⁺ or CD8⁺ TCR) or mice lacking costimulatory molecules, it was found that neither the first signal (antigen recognition) nor the second signal (costimulation) of classical T-cell activation was required for the T-cell-dependent damage to occur after stroke [264].

That is not to say that previously activated T lymphocytes, for example, due to preexisting infection or even cardiovascular disease [278], could not cause additional damage in the brain following I–R. The mechanism(s) of this antigen-independent T-cell ‘activation’ within hours after stroke are currently unknown. On the other hand, antibodies to neuroantigens reportedly increase following stroke, as do myelin basic protein reactive T cells [210–211]. Furthermore, another two studies reported that administration of the recombinant TCR ligand, RTL551 (which blocks classical antigen-dependent T-cell activation) linked to a CNS antigen, resulted in a reduced infarct volume following cerebral I–R [279].

Because this protective effect only occurred when RTL551 was linked to a neuroantigen, rather than a nonneuronantigen [57], it suggests that an adaptive immune response to brain antigens occurred following stroke, and that classical T-cell activation may indeed have contributed to postischemic brain damage. Moreover, tolerance against brain antigens by mucosal administration of the antigen before stroke has been reported to improve outcome after stroke [52, 56, 220], further suggesting that antigen-dependent lymphocyte activation occurs following stroke, and that it contributes to brain injury. Therefore, the contribution of

antigen-dependent T-cell activation in the damaging effects of T cells acutely poststroke remains unclear.

T-Lymphocyte-Targeted Experimental Therapies Anti- α 4 Integrin Antibody

Several studies have sought to prevent T-cell infiltration into the brain after stroke, for example, by targeting the α 4 integrin with neutralizing antibodies [280-281]. α 4 Integrin is part of the VLA-4 protein that is expressed on > 70% of all leukocyte populations, including T cells [209], and which binds to VCAM-1 expressed on endothelial cells. Vascular cell adhesion molecule-1 mRNA and protein [209, 229] are reported to be strongly induced in the microvasculature of the ischemic area following cerebral ischemia, and this expression is important for T-cell infiltration into the brain [282-83].

Furthermore, another study that treated mice with a monoclonal antibody against the α 4 integrin reported a reduced infarct volume at 7 days after a 30-minute I-R or permanent cerebral ischemia, as well as an improved sensorimotor activity 3 and 7 days after permanent ischemia [42]. This protection was confirmed to occur via limiting the effects of lymphocytes, as α 4 integrin blockade had no effect in Rag2^{-/-} mice. In addition, in both TH and TC cell-depleted mice, α 4 integrin blockade had no further effect on infarct volume, providing good evidence that the efficacy of α 4 integrin blockade is mediated by targeting T cells [203]. Interestingly, at 5 days after cerebral ischemia, the brain infiltration of all T-cell subsets examined (including TH, TC, Treg, and NKT cells) was significantly reduced, as were the infiltration of B cells and granulocytes. Together, these findings might suggest that even though multiple leukocyte types enter brain lesions after transient I-R, much of the (at least α 4 integrin inhibitable) damage is mediated by T cells.

Anti-Vascular Cell Adhesion Molecule-1 Strategies

The means by which VCAM-1 is targeted appears to be critical in the outcome achieved by anti-VCAM-1 therapy after stroke. Intravenous or intraperitoneal administration of anti-VCAM-1 antibodies have been unsuccessful at protecting against ischemic brain damage either in rats or in mice. While VCAM-1 blockade reduced the number of monocytes/macrophages/reactive microglia present in the ischemic rat brain, it did not reduce the numbers of infiltrating neutrophils and lymphocytes [284, 203]. In contrast, when utilizing hydrodynamic *in vivo* administration of VCAM-1 small interfering RNA, granulocyte and T-cell infiltration into the brain was reduced in association with a reduced infarct volume at 6 days after stroke. Moreover, after this *in vivo* silencing of VCAM-1, anti- α 4 integrin antibody administration produced no further reduction of infarct volume suggesting that VCAM-1 is the main endothelial receptor for VLA-4 on infiltrating T lymphocytes [203]. Interestingly, a very recent study found that a high plasma level of ALCAM in patients with acute ischemic stroke was predictive of a poor prognosis, raising the possibility that anti-ALCAM strategies might be considered clinically for acute stroke therapy [285].

FTY720

Another widely tested therapeutic in experimental stroke is the immunosuppressant FTY720, a stable analog of the lipid signaling mediator sphingosine 1- phosphate. Many experimental stroke studies have found reductions in infarct volume, and improvements in functional outcome [207-208, 286] following administration of FTY720, a drug known to sequester lymphocytes in lymph nodes, preventing them from moving to the CNS for autoimmune responses in multiple sclerosis. One study even found improvements in functional outcome up to 15 days after cerebral ischemia [208]. Shichita et al., (2009) found FTY720 to inhibit T-cell infiltration into the infarcted zone, and others have reported fewer infiltrating neutrophils and activated microglia/ macrophages in the ischemic lesion [51, 286-287].

Furthermore, FTY720 was found to reduce apoptotic cell death in the ischemic hemisphere as well as the expression of intercellular adhesion molecule-1-positive blood vessels in the brain. However, a recent study could not detect any reduction of infarct volume or behavioral dysfunction following permanent cerebral artery occlusion despite a reduced lymphocyte brain invasion after FTY720 treatment [203, 208]. This lack of neuroprotection despite effective lymphopenia was suggested to be due to a divergent impact of FTY720 on cytokine expression and possible activation of innate immune cells after brain ischemia [203].

FK506 and Cyclosporin A

Other immunosuppressive drugs, such as FK506 (Tacrolimus) and CsA (Cyclosporin A), have also been tested in experimental stroke studies. The immunosuppressive actions of FK506 and CsA involve the inactivation of NFAT (nuclear factor of activated T cells) via the inhibition of calcineurin. FK506 was first shown by Sharkey and Butcher (1994) to be a powerful neuroprotective agent in an in vivo model of focal cerebral ischemia when administered up to 60 minutes after occlusion. The minimum effective neuroprotective doses of FK506 and CsA are comparable with the immunosuppressant doses in humans, however, suggesting that a broad immunosuppressive effect predisposing to infection may complicate the clinical use of these drugs as a treatment for stroke [288-292].

RTL551

As mentioned above, two recent studies reported that postischemic administration of a recombinant TCR ligand (RTL551, which acts as a partial agonist at the TCR and blocks T-cell activation) linked to a CNS antigen, reduced cerebral infarct volume by up to 33% at 96 hours. These protective effects were associated with less brain infiltration by T cells, B cells, NK cells, and macrophages/microglia, and especially dendritic cells and activated microglia/macrophages [57], as well as fewer activated TH (CD44⁺CD4⁺) cells in the circulation [279].

Chemokine Receptor Antagonists

Chemokine receptors could be attractive novel targets for modulating T-cell-mediated damage poststroke. Chemokine receptors are expressed on leukocytes and, by binding with a high degree of specificity to chemokines released from damaged tissues, they promote the migration of leukocytes to sites of injury [293-294]. Several chemokines are upregulated in the brain following cerebral I-R in mice, underpinning the attraction of different leukocyte subtypes into the brain. We have provided proof-of concept that inhibition of chemokine/chemokine receptor interactions with a small molecule chemokine receptor antagonist (SB225002, a CXCR2 antagonist) can modulate the leukocyte profile in the brain following stroke [295]. We speculate that more detailed identification of the specific chemokine/chemokine receptor interactions that are involved in the attraction of damaging T-cell subsets (and any other leukocytes responsible for poststroke brain injury) may enable selective inhibition of their infiltration into the brain following stroke, without preventing the entry of those T-cell subsets that are involved in brain repair and regeneration (). Moreover, targeting T-lymphocyte migration into the brain following stroke (as opposed to targeting their activation) may have the added advantage of maintaining the levels of these cells in the periphery where they are needed to fight infections.

Evidence for T Lymphocyte Involvement in Brain Regenerative Processes after Stroke

Following the acute pathological events that occur in the hours to days after an ischemic stroke, regenerative processes associated with neurologic recovery take place in the brain, over several weeks or even months. During this process, the brain repairs and reorganizes in a manner similar to that which occurs during the early stages of development [296]. The creation of new neural networks through neurogenesis, neuroplasticity, synaptogenesis, angiogenesis, and gliogenesis helps to repair and reorganize the brain. Inflammation has been suggested to have a key role in the promotion of these reparative processes, mainly through the release of growth-related proteins and cytokines, potentially from T lymphocytes as well as other peripheral and resident immune cells. Neurogenesis occurs continually in the hippocampus throughout adult life [294], but when T cells are depleted, this neuronal cell proliferation is impaired, suggesting that T cells are required for neurogenesis [297-298]. This appears to be specifically due to the actions of CD4⁺ and not CD8⁺ T cells [297], and it occurs through classical CNS antigen-dependent T-cell activation [298] via the release of neurotrophic factors, such as nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 [299]. Thus, CNS-specific CD4⁺ T cells could be involved in the promotion of neurogenesis after brain injury, such as in stroke, and antigen-activated T cells at the site of injury could have a role in the repair of damaged tissue. By contrast, a recent study by Saino et al., (2010) reported that CD4⁺ T cells in the brain might inhibit neurogenesis following stroke. In mice depleted of CD4⁺ (but not CD8⁺) T cells, there was a greater proliferation of neurons at 28 days after stroke. However, it is important to note that these studies were performed using a model of permanent ischemia, and it is possible that the effect of T cells is different if reperfusion does not occur. Interestingly, when Treg cells are selectively depleted, neurogenesis is also reduced [300], suggesting that Treg cells may be a key CD4⁺

subpopulation of T cells that is required for neurogenesis. Moreover, Treg cells are also reported to modulate postischemic neovascularization following femoral artery ligation [254], and so any similar action by Treg cells to promote cerebral neovascularization after stroke would represent a further important role by this subset of T cells in the recovery of brain function following ischemia.

Complications and limitations to be considered in targeting T lymphocytes as poststroke therapy With tPA (tissue plasminogen activator) still being the only therapy available for acute ischemic stroke patients, novel effective therapies that can be administered beyond 4.5 hours are desperately needed. As acute inflammation appears to occur in the brain for many hours or even days after ischemic stroke [301], targeting key circulating immune cells to prevent their activation and/or extravasation would, at face value, seem to be a rational approach. Although therapies that target T cells in this manner relatively soon after stroke may reduce brain injury and improve poststroke outcome, there are several potential complications and likely limitations to the usefulness of T-cell inhibition after stroke.

First, in the short term, as the profound systemic immunodepression that rather quickly follows ischemic stroke will already have substantially weakened the immune system and thus increased the risk of infection [86,89, 303], further pharmacological approaches that attenuate immune cell function beyond this point (possibly within only a few hours) may do more harm than good by exacerbating the immunodepression. It should be noted that infection is the most common cause of death in the postacute phases of stroke with 23% to 65% of all stroke patients acquiring infections within the first few days [304-305].

Second, in the longer term (i.e., days to weeks) as mentioned above, inflammation—including via effects of T cells—appears to eventually have a role in promoting brain regeneration in the postacute phase of an ischemic stroke. For example, neurogenesis is reported to begin 5 days after stroke, and thus any therapy that interfered with these processes would be contraindicated.

Third, it is unclear how important T cells are as contributors to acute brain injury after ischemic stroke in the absence of effective reperfusion (either spontaneous or tPA induced), which still represents the majority of clinical cases. If their role is minimal, then anti-T-cell therapy may only be appropriate to be given in combination with successful reperfusion by tPA.

Fourth, the possibility of blocking T-cell subtypes with important antiinflammatory actions (e.g., Treg cells or NKT cells) would also be a limitation to its efficacy. More research is therefore needed to clarify the plausibility of safely and effectively inhibiting T-cell-mediated brain injury in the early stages after stroke, and perhaps even at later times using mild pro-inflammatory treatments (including neurotrophic factors) to promote repair and regeneration [306-307]. Additionally, the recent work by Wong et al., (2011) has raised the possibility of using selective activators of NKT cells to prevent stroke-associated infections [235].

SUMMARY AND CONCLUSION

Immunity and inflammation are an integral part of the pathogenic processes triggered by I/R. Inflammatory signaling is responsible for early molecular events triggered by the arterial

occlusion and culminating in the invasion of the brain by blood-borne leukocytes. Although its ultimate goal is to reestablish homeostasis, inflammation inflicts considerable damage to the metastable penumbral tissue. Adaptive immunity is deeply involved in the central and peripheral events triggered by cerebral ischemia, but evidence that a classical autoimmune response against brain antigens unveiled by tissue damage contributes to the acute phase of the damage is lacking. Lymphocytes invade the ischemic brain and contribute to tissue damage, but the rapidity of their deleterious effect is not consistent with an adaptive immune response targeted to the brain. Remarkably, selected lymphocyte subpopulations are protective, acting to dampen the cytotoxic effects of other inflammatory cells and promoting tissue recovery. The mechanistic bases for this dichotomous response of lymphocytes remains to be elucidated (Figure 3). Although the participation of inflammation and immunity in tissue recovery is well established in other organs, very little is known about these processes in the brain. Even less clear are the long-term effects of the adaptive immune response associated with stroke, and their role in the sequelae of ischemic damage, such as brain atrophy and dementia. Similarly, it is unclear if the immune system develops a memory of the antigen exposure and if an autoimmune response develops following subsequent exposure to the same antigen, as it may occur in cases of recurrent strokes.

The realization that the immune system and inflammation are central to the pathophysiology of stroke has raised the prospect of new therapeutic approaches to counteract ischemic injury. However, our understanding of the crosstalk between the immune system and the ischemic brain is still rudimentary and, as suggested by failed clinical trials, not adequate to guide therapeutic interventions. Modulation of adaptive immunity may afford the opportunity to deviate the post-ischemic immune response away from tissue damage and towards protection, an approach very effective in stroke models. However, immune-modulation can also have deleterious effects that need to be considered. Nevertheless, the remarkable impact that modulation of the immune system has on stroke damage and repair, justifies the aggressive pursuit of basic and clinical investigations seeking to unravel the fundamental processes governing the interaction of the ischemic brain with the immune system.

Innate immune system, triggered immediately and kept for a while after ischemic stroke onset, protects and hurts brain by activation of endogenous and exogenous immune cells and production of cytokines. Immunomodulatory therapies targeting the poststroke inflammation are promising with great obstacles, and a comprehensive understanding of innate immune response to cerebral ischemic attack calls for further investigation.

The relationship between the CNS and the immune system is complex and remains incompletely understood. It has particular salience after stroke and other forms of CNS injury, which trigger immune processes that seem to be both beneficial and harmful. A major frontier in stroke research involves efforts to better understand these interactions to develop new strategies and drugs that will prevent and reduce the burden of stroke. Based on current knowledge, physicians should be mindful that underlying inflammation is a biomarker of stroke risk and should carefully consider antithrombotic, statin, and antihypertensive therapy in vulnerable populations. Further work will be needed to delineate precise clinical strategies for risk factor modification based on specific biomarkers. In addition, it would be reasonable to administer statin drugs to patients with acute stroke given data suggesting that this improves outcomes, possibly as a result of anti-inflammatory properties. Furthermore, physicians caring for patients with stroke should recognize that poststroke immunodepression

increases the risk of infection and should adjust their clinical suspicion and treatment strategies accordingly. Whether a strategy of routine prophylactic antibiotic administration after stroke is beneficial remains unknown, but it holds promise as a simple method for improving poststroke outcomes. Finally, the care of patients with stroke may be improved by advances in specific areas, including investigation of whether modulating inflammatory pathways can reduce the risk of stroke and decrease penumbral ischemia during acute stroke, whether immunity has a role in poststroke functional recovery and dementia, and whether strategies to prevent poststroke immunodepression can reduce the incidence of infection after stroke without increasing dangerous autoimmunity against the brain. The immune system has not traditionally been the subject of therapeutic manipulation in patients with stroke, but given its intertwined relationship with the CNS, it promises to be an exciting avenue for future attempts to reduce the high burden of disability and death from stroke.

The study of the immune system is receiving increasing attention in the field of stroke, and detailed knowledge of the complex interactions between the innate and adaptive immune systems should improve our understanding of the disease course and the increased risk of infections after stroke. Emerging data are confirming, both at the bench and at the bedside, the influence of neurohormonal factors and the autonomic nervous system on the risk of poststroke infection. Studies in animal models have highlighted the involvement of immune cells that are resident in peripheral organs, such as the liver, in modulating infection after stroke—a phenomenon that needs to be investigated in patients in future studies. Similarly, further studies are required to address the apparently heterogeneous clinical consequences of the immune system responses that are associated with different cellular compartments of the CNS. Clarification of the mechanisms that produce the benefits and harmful effects of different lymphocyte subpopulations will also be an essential step in developing immunomodulatory therapies for acute stroke. In summary, this Review emphasizes the double-edged nature of the crosstalk between the CNS and the immune system in acute stroke, and highlights the strong need for further translational research.

In the last 5 years or so, there have been substantial advances in our understanding of the pathogenic role of T lymphocytes in ischemic stroke. These cells, traditionally belonging to the adaptive immune system, are now established to contribute to the infarct volume in the postischemic brain. Current evidence suggests that following an ischemic stroke, a systemic immunodepression occurs, T lymphocytes enter the brain and release cytokines/chemokines and superoxide, and contribute substantially to neuronal injury. Importantly, some types of T cells may be beneficial (e.g., Treg cells and NKT cells), and T cells may also have a longer-term role in general regenerative processes that occur in the later stages following an ischemic stroke. T lymphocytes may produce their damaging acute effects following activation via a nonclassical, antigen-independent process but this point is still controversial. After 7 to 10 days, T lymphocytes may additionally become activated in the classical manner, producing neurotrophins and contributing to neurogenesis. In conclusion, the role of T lymphocytes in ischemic stroke is complex and remains poorly understood. More research is needed to gain a greater understanding of which T-cell subpopulations produce the most damage, how and when this occurs, and when T cells may cease causing damage and begin contributing to regeneration. With this information, it will become apparent whether therapeutic targeting of T lymphocytes is likely to offer benefits for stroke patients.

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Chapter 18

NATURAL HERBS, HUMAN BRAIN AND NEUROPROTECTION

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ABSTRACT

A considerable amount of research has been invested into the development of novel treatments capable of protecting the brain from damage following stroke, with limited success. In ischemic stroke, the primary focus of treatment is reperfusion. Currently, the only drug approved for the treatment of ischemic stroke is recombinant tissue plasminogen activator (rtPA, alteplase), which has a limited time window for administration and increases the risk for subsequent hemorrhage. Humans consume a wide range of foods, drugs, and dietary supplements (Phytochemicals) that are derived from plants and which modify the functioning of the central nervous system (CNS). This chapter assesses the current evidence for the efficacy of a range of readily available plant-based extracts and chemicals that may improve brain function and which have attracted sufficient research in this regard to reach a conclusion as to their potential effectiveness as nootropics. Many of these candidate phytochemicals/extracts can be grouped by the chemical nature of their potentially active secondary metabolite constituents into alkaloids (caffeine, nicotine), terpenes (ginkgo, ginseng, valerian, *Melissa officinalis*, sage), and phenolic compounds (curcumin, resveratrol, epigallocatechin-3-gallate, *Hypericum perforatum*, soy isoflavones). They are discussed in terms of how an increased understanding of the relationship between their ecological roles and CNS effects might further the field of natural, phytochemical drug discovery. Currently there are no approved treatments for the myriad of damaging pathological processes that persist in the brain long after the acute stage of ischemic injury. These include the processes of inflammation, excitotoxicity, oxidative stress, apoptosis, and edema resulting from disruption of the blood brain barrier. In hemorrhagic stroke, additional processes include physical damage from the mass of accumulated blood itself, cytotoxicity of blood

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components, and vasospasm in subarachnoid hemorrhage. In spite of decades of focused research on stroke treatment options still remain limited. Numerous neuroprotective treatments have been identified that show great promise in animal models of stroke. Natural compounds with the effects of anti-oxidation, anti-inflammation, calcium antagonization, anti-apoptosis, and neurofunctional regulation exhibit preventive or therapeutic effects on experimental ischemic brain injury. The prevention of cerebrovascular diseases has been one of the primary goals of researchers but unfortunately, to date, no such safe preventive agents are available. There is an urgent need for agents that are pharmacologically safe, cost-effective, and immediately available with minimal side effects. Here in this chapter, we will discuss the promising targets of neuroprotection and the natural products from traditional medicinal herbs that exhibit protective effects on ischemic brain injury. Further, we will provide an overview of targets for neuroprotection in stroke and examples of current research on potential neuroprotective treatments.

INTRODUCTION

Cerebral ischemia, which is one of the leading causes of death and disability worldwide, has attracted more and more attention in the field of drug discovery. Stroke is often caused by transient or permanent reduction of cerebral blood flow initiated by thrombotic or thromboembolic arterial occlusions [1-2]. The primary focus of treatment in stroke is reperfusion. Currently, the only drug approved for the treatment of ischemic stroke is recombinant tissue plasminogen activator (rtPA, alteplase), which has a limited time window. Thrombolytic therapy designed to restore cerebral perfusion in a timely fashion is considered the main rational therapeutic strategy for ischemic brain injury [3]. However, reperfusion after thrombolytic therapy often leads to a series of cellular, biochemical and metabolic consequences of cerebral ischemia, including intracellular reactive oxygen species (ROS) generation, calcium overload, excitotoxic cell injury and inflammation, which ultimately lead to irreversible brain injury. Moreover rtPA increases the risk for subsequent hemorrhage. Consequently, only a small percentage of patients receive rtPA treatment [3]. While this treatment is effective in opening up occluded cerebral vessels in some patients and can lead to improved outcomes after ischemic stroke, there are currently no approved treatments for the myriad of damaging pathological processes that persist in the brain long after the acute stage. These include the processes of inflammation, excitotoxicity, oxidative stress, apoptosis, and edema resulting from disruption of the blood brain barrier. Many neuroprotective agents are designed to protect the brain from irreversible injury after ischemia-reperfusion or to retard the pathological process [4, 5].

Recent studies have shown that damage to neurons after ischemia/reperfusion may occur through oxidative stress, inflammation and/or mitochondrial impairment that may culminate in the activation of an apoptotic cell death. Therefore, great emphasis has been given for the development of antioxidant, anti-inflammatory and antiapoptotic agents as therapeutics for stroke. Numerous lines of evidence from *in vitro* and *in vivo* studies have shown that plant-derived nutraceuticals have potential as antistroke agents.

Currently, research studies have focused on the possible capacity of natural herbs and the compounds extracted from fruits, vegetables and beverages to prevent ischemia and other neurological diseases. Some beneficial phytochemicals, display protective abilities in various

animal models of stroke and neurological disorders. In fact, many effective components extracted from traditional herbs have been demonstrated to show neuroprotection against ischemic brain injury in experimental studies. According to the pharmacological mechanisms elucidated in numerous reports, we evaluated the natural products that possess protective effects on ischemic brain injury and characterized the promising targets for ischemic brain injury.

But, despite decades of research, treatment options remain limited. Therefore, more and more attention in the field of drug discovery has been focused on neuroprotection by natural compounds from traditional medicinal herbs. Cerebral ischemia is a complex pathological process involving a series of mechanisms, and a framework for the development of neuroprotectants from traditional herb medicine is a promising treatment for cerebral ischemia.

Herbs that affect the functioning of Human Brain and provide neuroprotection

Humans consume a wide range of foods, drugs, and dietary supplements that are derived from plants and which modify the functioning of the central nervous system (CNS). The psychoactive properties of these substances are attributable to the presence of plant secondary metabolites, chemicals that are not required for the immediate survival of the plant but which are synthesized to increase the fitness of the plant to survive by allowing it to interact with its environment, including pathogens and herbivorous and symbiotic insects. In many cases, the effects of these phytochemicals on the human CNS might be linked either to their ecological roles in the life of the plant or to molecular and biochemical similarities in the biology of plants and higher animals. This section of the chapter assesses the current evidence for the efficacy of a range of readily available plant-based extracts and chemicals that may improve brain function and which have attracted sufficient research in this regard to reach a conclusion as to their potential effectiveness. Many of these candidate phytochemicals/extracts can be grouped by the chemical nature of their potentially active secondary metabolite constituents into alkaloids (caffeine, nicotine), terpenes (ginkgo, ginseng, valerian, *Melissa officinalis*, sage), and phenolic compounds (curcumin, resveratrol, epigallocatechin-3-gallate, *Hypericum perforatum*, soy isoflavones).

A huge scientific literature focusing on psychoactive herbal extracts and their phytochemicals, encompassing hundreds of thousands of scientific papers, has emerged over recent decades. The vast majority of these papers describe *in vitro* investigations of the potential mechanisms of action of putatively psychoactive phytochemicals, whereas a much smaller proportion explores their effects *in vivo* in animals and only a tiny minority investigate their efficacy in humans.

A vast number of natural, plant-based extracts and chemicals are purported to have beneficial effects on human brain function. Zhang identified extracts and constituents from 85 individual medicinal plants that have demonstrated potential efficacy for treating psychiatric disorders on the basis of animal behavioral models alone [6]. However, few plant-based products have been assessed in methodologically adequate human trials. A simple literature search using the individual names of the few extracts and compounds reviewed below (excluding nicotine and caffeine) generates some 30,000 publications. The 3 single polyphenols [epigallocatechin-3-gallate (EGCG), curcumin, and resveratrol] alone contribute 15,000 of these papers, the vast majority of which have been published in the last 10 yrs. This represents a huge amount of research and naturally raises the question of the ultimate efficacy

of the interventions in question. The following comprises a brief outline of the evidence surrounding the handful of herbal extracts and phytochemical supplements that have garnered enough evidence of efficacy or have been subjected to adequate levels of research to allow any conclusion as to their efficacy in terms of improved brain function. The polyphenols are included on the basis of the enormous interest they are generating currently. The palette of secondary metabolites can be subdivided into a number of distinct groups on the basis of their chemical structure and synthetic pathways, and these groups can, in turn, be broadly differentiated in terms of the nature of their ecological roles and therefore their ultimate effects and comparative toxicity in the consuming animal. The extracts/phytochemicals are therefore grouped below by the chemical nature of their putative active components. In this regard, the largest and most prevalent of phytochemical groups are the alkaloids, terpenes, and phenolic compounds.



Figure 1. Natural Herbs (Spices) with potential against Cerebrovascular diseases.

Numerous lines of evidence indicate that chronic inflammation plays a major role in the development of various cerebrovascular diseases. Extensive research over the last 10 years has indicated that nutraceuticals derived from such spices as turmeric, red pepper, black pepper, licorice, clove, ginger, garlic, coriander, and cinnamon target inflammatory pathways, thereby may prevent cerebrovascular and neurodegenerative diseases. The prevention of cerebrovascular diseases has been one of the primary goals of researchers, but to make prevention feasible, two objectives must be accomplished: (a) individuals at high risk for the disease must be identified before the symptoms become evident, and (b) compounds that are safe and effective in either reducing or slowing the disease progression need to be developed. Unfortunately, to date, no such safe preventive agents are available. There is an urgent need for agents that are pharmacologically safe, cost-effective, and immediately available with minimal side effects. Natural herbs like spices are one such source that has been used in cooking to add flavor and color to the food. A spice is a dried seed, fruit, root, bark, or flower of a plant. The use of spices has shaped a large part of the world's history. For example, the ancient Egyptians pioneered maritime trade to fetch the incense of Arabia; Greco-Roman navigators found their way to India for pepper and ginger; Columbus sailed west for spices; Vasco de Gama sailed east for them; and Magellan sailed across the Pacific Ocean on the same quest. Despite globalization, persons in Asian countries are still the largest consumers of spices. In ancient times, many spices were used as medicines for treating several diseases such as rheumatism, body ache, intestinal worms, diarrhea, intermittent fevers, hepatic diseases, urinary discharges, dyspepsia, inflammation, constipation, and dental diseases [7–8].

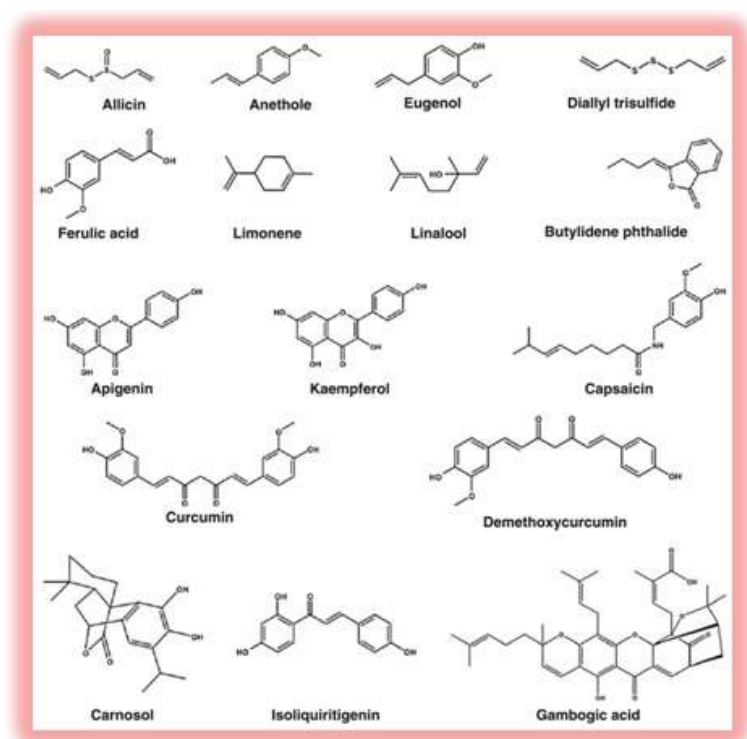


Figure 2. Chemical structure of common nutraceuticals derived from natural herbs (spices).

What was in such spices and how they exerted these activities remained obscure for ancient peoples. Modern molecular tools have shown that spices have active components, called nutraceuticals that contribute to the plethora of properties. Extensive research over the years has also identified the molecular targets of most nutraceuticals [9-10]. During the past decade, a number of nutraceuticals have been identified from spices (Figure 1). These nutraceuticals are chemically diverse (Figure 2) with a plethora of effects [11].

TARGETS FOR NEUROPROTECTION IN STROKE

Natural compounds with the effects of anti-oxidation, anti-inflammation, calcium antagonization, anti-apoptosis, and neurofunctional regulation exhibit preventive or therapeutic effects on experimental ischemic brain injury. Here in this chapter we will discuss the promising targets of neuroprotection and the natural products from traditional medicinal herbs that exhibit protective effects on ischemic brain injury. Further we will provide an overview of targets for neuroprotection in stroke and examples of current research on potential neuroprotective treatments.

Oxidative Stress as a Target for Neuroprotection in Stroke

The production of reactive oxygen species (ROS) and other free radicals during stroke is a consequence of excitotoxicity and the inhibition of cellular respiration in a low oxygen environment in addition to inflammation [12]. These molecules, such as hydroxyl radical, superoxide, and peroxynitrite, are highly reactive and damaging to multiple cellular components, leading to cell death (Figure 3). One way of reducing oxidative stress is to reduce the production of free radicals. Although nitric oxide is a normal signaling molecule in the body and has beneficial effects in stroke, larger amounts resulting from increased activity of the induced nitric oxide synthase (iNOS) can lead to aberrant signaling and or react with superoxide to produce peroxynitrite. Nebivolol decreases the expression of iNOS following bilateral CCAO in rats and increases expression of the beneficial endothelial nitric oxide synthase (eNOS), leading to a reduction in histopathological changes [13]. Another source of ROS is the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, and inhibitors of these enzymes could be beneficial in stroke [14]. Another method of protection is to induce endogenous mechanisms for the removal of free radicals in the body. Hydrogen sulfide gas increases the activity of superoxide dismutase and glutathione peroxidase in rats subjected to focal cerebral ischemia, resulting in decreased injury to neuronal mitochondria and a subsequent reduction in markers of apoptosis [15]. Hydrogen-rich saline increases endogenous antioxidant enzyme activity and decreases the amount of oxidative products in pMCAO rats [16]. A nucleic acid-based product improves the antioxidant status of neuronal mitochondria after transient ischemia in rats [17]. Alternatively, exogenous compounds with free radical scavenging properties can be used. The novel compound MnTm4PyP mimics the activity of endogenous manganese superoxide dismutase, and reduces infarct volume and neurological deficit after MCAO in mice [18]. An extract of *Ocimum sanctum* protects tMCAO rats by preserving reduced glutathione content and antioxidant enzyme activity [19]. Hydrogen gas

has been shown to neutralize free radicals and has beneficial effects on several contributors to early brain injury after subarachnoid hemorrhage in rats [20]. Furthermore, non-effective doses of the free radical scavenger lipoic acid enhance the neuroprotective effects of the NADPH oxidase inhibitor apocynin when given in combination to tMCAO rats [21]. Inhibition of downstream signaling pathways leading to oxidative stress induced damage, rather than the direct removal of ROS, may also have beneficial effects. The antioxidant N-tertbutyl- α -phenylnitron suppresses expression of complement component 3, a mediator of inflammation that is induced by oxidative stress, in mice after transient focal cerebral ischemia [22].

Natural Compounds with Anti-Oxidative Properties

Reactive oxygen species (ROS) is produced during ischemia/reperfusion, leads to the oxidation of lipids, proteins and DNA and subsequently cellular damage and apoptosis has now been well established [23-24]. Therefore, much attention has been paid to the rescue of brain injury after ischemia/ reperfusion via inhibition of ROS bursts. In fact, many natural compounds with antioxidant ability, such as flavonoids from *Scutellaria baicalensis* Georgi[25], Carnosic acid (CA), found in the herb rosemary obtained from *Rosmarinus officinalis*[26], Curcuma Oil (isolated from powdered rhizomes of *Curcuma longa* Linn)[27], *Ginkgo biloba* extract EGb761[28], and Cinnamophilin (isolated from *Cinnamomum philippinense*) [29], exhibit significant neuroprotective effects when they are administered before cerebral ischemia occurs, but the related mechanisms or targets have been identified for only a few. For instance, flavonoids from *Scutellaria baicalensis* Georgi, when either pretreated or post-treated, are demonstrated to decrease levels of malondialdehyde (MDA) and increase the level of superoxide dismutase (SOD) in the ischemic brains of mice. Aside from the anti-oxidant effects, flavonoids are also found to inhibit platelet aggregation, which is important to improve ischemic brain injury [25]. Pretreatment with curcuma oil, isolated from powdered rhizomes of *Curcuma longa* Linn, significantly reduces the levels of NO, ROS, ONOO⁻, and mitochondrial membrane potential [27].

Natural antioxidant compounds from various herbs protecting against cerebral ischemia have been reported in many recent studies. Some natural compounds exhibit direct regulatory effects on endogenous antioxidant enzyme systems. For example, Heme oxygenase (HO) is the rate-limiting enzyme for catabolism of the prooxidant heme. Two isoforms of HO exist: an inducible form (HO-1) and a constitutively expressed form (HO-2). HO-1 can be induced in response to various noxious stimuli (such as hypoxia and oxidative stress) and is considered a gene that protects against I/R injury[30-32]. CA, a catechol-type electrophilic compound found in the herb rosemary obtained from *Rosmarinus officinalis*, is shown to be neuroprotective when injected 1 h prior to MCAO in mice. As a representative electrophile, CA can induce the expression of a set of antioxidant enzymes, including heme oxygenase-1 (HO-1), NADPH quinone oxidoreductase 1 (NQO1), and c-glutamyl cysteine ligase (c-GCL). CAs become electrophilic quinones upon oxidation, with protective effects against neuronal oxidative stress and excitotoxicity via binding to specific Keap1 cysteine residues as a direct drug target, and then activating the Keap1/Nrf2 transcriptional pathway. The most attractive advantage of this agent is that it is a pro-electrophilic compound that can be activated by the microenvironment of oxidative stress and only becomes electrophilic at or near the site of ischemic brain tissues, with lower toxicity to normal tissues [26]. The protective effect of *Ginkgo biloba* extract on cerebral ischemia can be abolished in HO-1 knockout mice,

suggesting that HO-1 is the key target of neuroprotection against free radical damage [2, 28]. In cerebral ischemia, nitro oxide (NO) plays both a protective and a destructive role at different stages of this complex process. The beneficial effects of NO are related to the small amount of NO produced by endothelial nitric oxide synthase (eNOS), which produces significant effects on the maintenance of cerebral blood flow, prevention of neuronal injury by activation of the GC-cGMP-PKG pathway, and inhibition of platelet as well as leukocyte adhesion, and therefore protects against cerebral ischemia[33-34]. Refined Qing Kai Ling (RQKL), an improved injectable multi-component preparation derived from Qing Kai Ling, shows neuroprotection in MCAO rats by relieving vascular endothelial cell damage as well as inhibiting inflammation. More importantly, RQKL was able to stimulate the post-ischemic expression of eNOS, which might be an essential part of the neuroprotective mechanisms of RQKL [35]. However, the large amount of NO, which is derived from inducible nitric oxide synthase (iNOS), harms neurons by producing peroxynitrite after the reaction with superoxide. Peroxynitrite can inhibit the mitochondrial respiratory chain, which implicates the involvement of ATP loss and eventually leads to irreversible cellular damage [36-37]. Tetrahydroxystilbene glucoside (TSG), an active component of the rhizome extract from *Polygonum multiflorum*, has been reported to attenuate intracellular ROS generation and mitochondrial membrane potential dissipation caused by ischemia/ reperfusion. Interestingly, it can directly upregulate the expression of sirt1, which is a class III histone deacetyltransferase that promotes cell survival and subsequently reduces the expression and activity of iNOS. This in turn induces the increase in NO production as well as peroxynitrite formation and results in apoptotic cell death by inhibiting the phosphorylation and subsequent degradation of I- κ B, thereby hampering the DNA binding of nuclear factor kappa-B (NF- κ B) by sirt1 activation [38]. More evidence has emphasized the significance of sirt1 in promoting cell survival, regulating lifespan and inhibiting inflammation. Recently, sirt1 has been introduced for the therapy of neurodegenerative diseases [39-40].

The natural compounds like as resveratrol, butein and quercetin, which are known as anti-aging agents, have been found to directly activate sirt1[41], suggesting that sirt1 may be the direct target of many herbal components that exhibit anti-aging effects. Phosphoinositide 3-kinases (PI3K)/Akt regulate the survival response against oxidative stress-associated neuronal apoptosis, which is determined by the balance between the activity of PI3K and the phosphatase and tensin homolog (PTEN). Activation of Akt promotes cell survival and suppresses apoptosis by inhibition of several downstream substrates, including glycogen synthase kinase-3 β (GSK3 β). PTEN is a major negative regulator of the PI3K/Akt signaling pathway and has been demonstrated to act as an important mediator of ROS production and mitochondria-dependent apoptosis [42]. A recent study reveals that Baicalein (Bai), one flavonoid extracted from *Scutellaria baicalensis* Georgi, when administered either prior to or after ischemia, can significantly protect against brain injury. Similarly, incubation with Bai reverses the rapid PTEN dephosphorylation after oxygen glucose deprivation (OGD) in cultured hippocampal neurons. Npg PTEN siRNA largely abolished the protection of Bai against OGD-induced cell injury, which highlighted the critical role of PTEN signaling in Bai-mediated effects [43]. The cytoplasmic enzyme NADPH oxidase is another important target for ROS production in cerebral ischemia and has received increased attention in recent years. Excessive activation of the neuronal *N*-methyl-*D*-aspartate receptor (NMDAR) initiates superoxide formation and promotes neuronal death. Previous studies have suggested that mitochondria are the primary source of NMDAR-induced superoxide production, but there is

no definitive evidence. A recent study revealed that activation of NADPH oxidase is required for NMDAR-mediated superoxide production [44]. Interestingly, some natural compounds inhibit NADPH oxidase. For instance, sinomenine, an alkaloid extracted from a Chinese medicinal plant, *Sinomenium acutum*, which has been used widely in the clinic, apparently inhibits the activation of microglial NADPH oxidase [45]. The ethanol extract of *E rutaecarpa*, an anti-inflammatory drug commonly used in traditional Chinese medicine includes four bioactive compounds (dehydroevodiamine, evodiamine, rutaecarpine, and synephrine) and exhibits antioxidative effects by inhibiting NADPH oxidase activity [46]. These novel medicinal targets and mechanisms provide innovative clues and can help researchers to screen drugs for therapeutic intervention in ischemic brain injury mediated by natural antioxidants. Some natural antioxidant compounds exhibit therapeutic effects on brain ischemia in the clinic. For example, cinnamophilin, extracted from *Cinnamomum philippinense*, has been demonstrated to reduce brain infarction and improve neurobehavioral outcome when administered either 15 min before (pretreatment) or 2 h after the onset of middle cerebral artery occlusion (MCAO) (posts ischemic treatment)[29]. TSG also protected against brain injury at 2 h after cerebral ischemia [22]. A significant effect of curcuma oil (500 mg/kg body wt) given 4 h postischemia was also observed in a rat MCAO model [47]. These findings offer beneficial references for the application of natural antioxidants in clinical treatment of ischemia and related neuronal diseases.

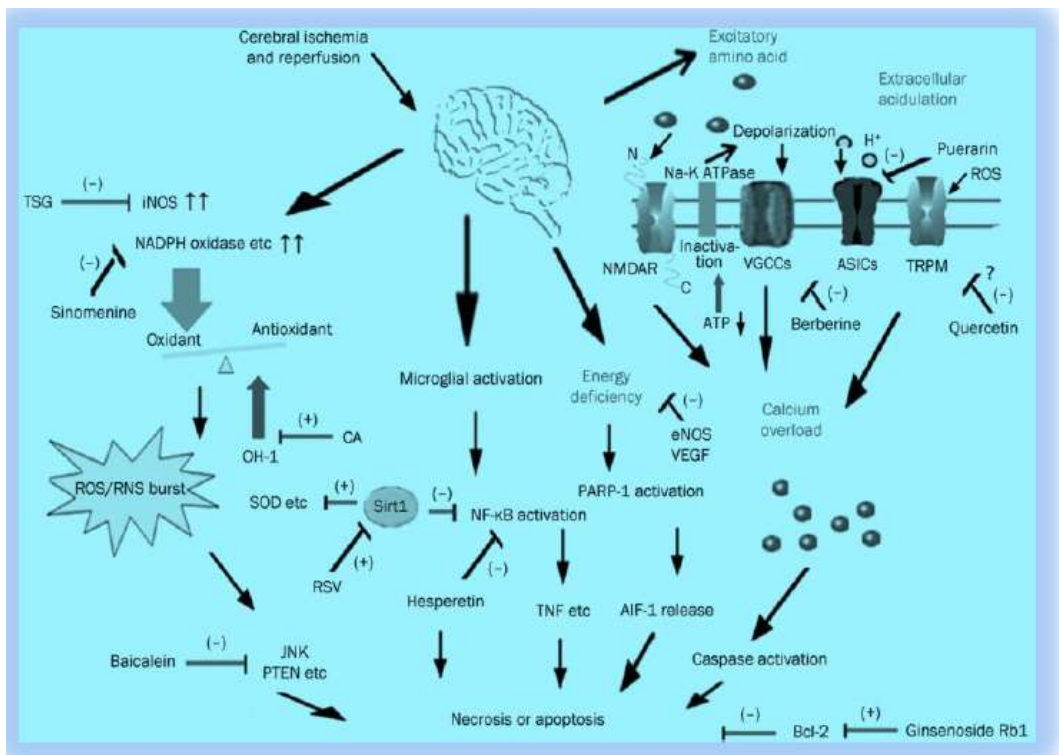


Figure 3. Multiple signal pathways and potential targets involved in the neuroprotection of natural compounds.

Inflammation as a Target for Neuroprotection

Inflammation is obvious within several hours during ischemia/ reperfusion injury; it contributes to secondary damage caused by the microglial activation and resident perivascular and parenchymal macrophages, as well as infiltration of peripheral inflammatory cells [2]. A significant amount of the research on neuroprotection following stroke is concentrated on mitigating the effects of inflammation. An overview of the inflammatory process in the brain after stroke is shown in (Figure 4). Following ischemia and reperfusion, damaged brain tissue secretes cytokines and chemokines that recruit inflammatory cells to the injured area [48]. These cells release their own secretory factors, which can build up to toxic levels. Inflammatory processes also result in the production of reactive oxygen species, leading to oxidative stress and activation of matrixmetalloproteinases (MMPs), causing disruption of the blood-brain barrier (BBB) and edema.

On the other hand, inflammation has beneficial effects as well, such as increasing blood flow to the affected area and the removal of damaged tissue by phagocytic cells and MMPs. The positive versus negative effects of inflammation following stroke and the appropriateness of intervention are a topic that is often debated [49]. It is generally considered, however, that inflammation does more harm than good after stroke, especially in the early stages. One important molecule resulting in cell damage and death following stroke is tumor necrosis factor alpha (TNF α). TNF α interacts with two receptors, R1 and R2, that mediate death signals via the Fas associated death domain (FADD) and inflammation via the nuclear factor kappa-light-chain enhancer of activated B cells (NF κ B), respectively [50]. Activation of the NF κ B pathway is commonly used as an indicator of inflammation in stroke studies. The interleukins are another important set of molecules in the process of inflammation. Interleukin-1 (IL-1) is proinflammatory, whereas IL-10 is anti-inflammatory and IL-6 has both pro- and anti-inflammatory effects [51]. Antagonists of the IL-1 receptor have been shown to be neuroprotective when administered at reperfusion in comorbid tMCAO rats [52].

As one of the early initiators of inflammation after stroke, TNF α is an excellent target for neuroprotective treatments. Perhaps the most straightforward way to block the effects of TNF α is to prevent or reduce its production. The thalidomide analog 3,6 -dithiothalidomide (3,6 -DT) is an inhibitor of TNF α synthesis that has been shown to reduce the number of activated inflammatory cells in the brain after ischemic stroke in mice, as well as the extent of BBB disruption [53]. Caffeic acid ester fraction reduces infarct volume and improves performance on behavioral tests in rats subjected to MCAO [54]. Further experiments with cultured microglia suggest that this effect is due to inhibition of the production of TNF α , as well as nitric oxide (NO) and IL-1 β . Atorvastatin suppresses TNF α levels in a rat model of intracerebral hemorrhage, reducing brain water content and activation of microglia [55]. Another method of action against TNF α is the use of decoy receptors. Fusion proteins consisting of TNF receptor linked to a monoclonal antibody are capable of crossing the blood-brain barrier and significantly reduce infarct volumes after tMCAO in mice [56].

Activation of NF κ B by TNF α initiates a signaling cascade that regulates a number of inflammatory processes, making it a good point of intervention. Honokiol has been shown to suppress the activation of NF κ B in ischemic mice as well as levels of TNF α and significantly reduces brain water content [57]. Rosmarinic acid blocks activation of NF κ B by TNF α after tMCAO in diabetic rats and reduces edema and tissue damage [58]. Suppression of NF κ B

activity by angiotensin-(1–7) reduces infarct volumes, improves neurological deficits, and decreases oxidative stress in rats subjected to pMCAO [59]. Kaempferol glycosides inhibit the activation of NF κ B as well as the signal transducer and activator of transcription 3 (STAT3), another proinflammatory transcription factor, in tMCAO rats, resulting in reduced infarct volume and neurological deficits [60]. It should be noted that not all NF κ B activity is harmful, and harmful activation of the NF κ B complex is associated with abnormal acetylation of the RelA subunit. Using a combination of an inhibitor of one type of deacetylase and an activator of another, it is possible to produce a RelA acetylation similar to that seen in the beneficial phenomenon known as ischemic preconditioning, resulting in neuroprotection in mice exposed to tMCAO [61].

The various signaling cascades induced by stroke lead to the activation and recruitment of inflammatory cells to the site of injury. In the early stages of stroke, prior to the infiltration of neutrophils and macrophages from other locations, resident microglia are the primary inflammatory cells in the brain. Microglia continue to be involved well into long term recovery and have been observed 28 days following stroke in MCAO rats [62]. Although microglia serve a beneficial purpose by removing dead tissue, they also release secretory factors that can accumulate to toxic levels, particularly in cases of excess activation such as stroke.

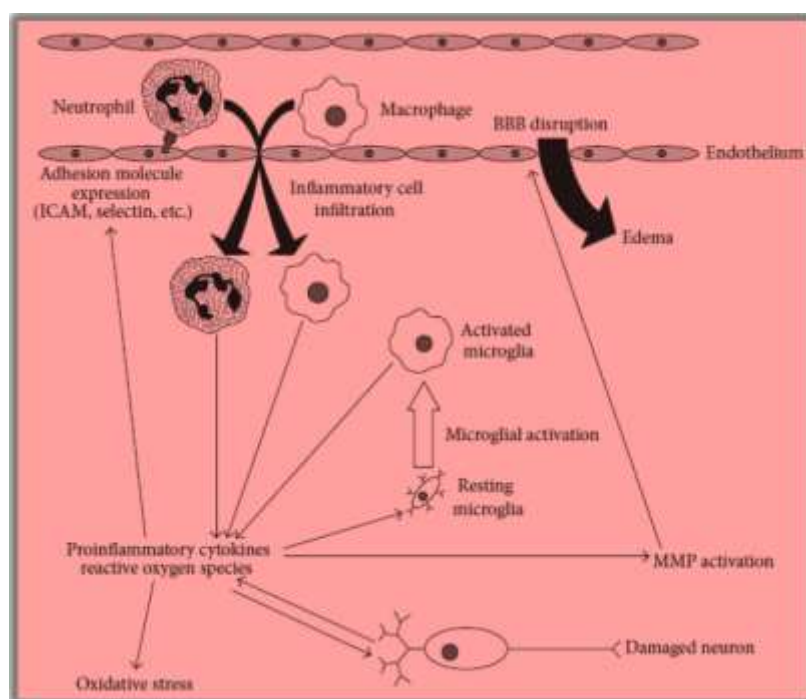


Figure 4. Damaging inflammatory mechanisms in stroke. Proinflammatory cytokines and reactive oxygen species released by damaged neurons lead to the activation of microglia and the expression of cellular adhesion molecules on endothelial cells and migrating inflammatory cells. Infiltrating inflammatory cells and activated microglia secrete additional cytokines and oxygen species, resulting in further tissue damage, oxidative stress, and activation of matrix metalloproteinases leading to disruption of the blood-brain barrier and edema.

Accordingly, treatments that limit microglial activation often have neuroprotective effects. The ginseng metabolite compound K suppresses microglial activation by inhibiting multiple upstream signaling molecules and is neuroprotective in MCAO mice [63]. Sesamin is neuroprotective in a mouse model of ICH and has been shown to prevent an increase in microglial cells by keeping them in their resting state [64].

Retinoids are also neuroprotective in models of ICH and reduce levels of activated microglia even with posttreatment [65]. Alternatively, increasing the reactivity of microglia can also have neuroprotective effects. The ATP-dependent potassium channel blocker glibenclamide increases the phagocytic capacity of microglia, resulting in improved neurological outcome, reduced infarct volume, and enhanced neurogenesis in rats subjected to transient or permanent MCAO [66-68]. Activation of the microglial α -7 nicotinic acetylcholine receptor induces expression of the heme oxygenase-1 (HO-1) gene, which is associated with neuroprotection in mice after photothrombotic stroke [69].

The processes involved in inflammation may not only directly contribute to brain damage following stroke but may also activate secondary mechanisms that lead to further damage. The activity of large numbers of inflammatory cells in the affected area, combined with low oxygen and ATP levels, leads to the formation of reactive oxygen species and the onset of oxidative stress. Activation of MMPs, while important for the removal of dead tissue and the ability of immune cells to enter the brain, may also result in disruption of the blood-brain barrier and edema due to an influx of water. These topics will be discussed separately in the following sections.

Natural Compounds with Anti-Inflammatory Effects

Inflammation is obvious within several hours during ischemia/ reperfusion injury; it contributes to secondary damage caused by the microglial activation and resident perivascular and parenchymal macrophages, as well as infiltration of peripheral inflammatory cells. Aside from regulation of inflammation at the molecular level, progressive ischemic brain injury is related to several post-injury inflammatory responses at the cellular level, including neutrophil response as early as 4 h after ischemia/reperfusion and delayed macrophage infiltration, which occurs several days later [2]. Sufficient evidence has indicated that neutrophils play a key role in the development of ischemic brain damage, and the depletion of circulating neutrophils or inhibition of neutrophil infiltration is demonstrated to ameliorate ischemic cerebral injury [70]. Thus, more and more evidence suggests that anti-inflammatory treatment might reduce ischemic brain injury and facilitate recovery [71]. Many bioactive components from Chinese medicinal plants exhibit significant anti-inflammatory effects, and recent studies have revealed that some exhibit neuroprotection against cerebral ischemia. Such compounds include theaflavin [72], Wogonin (a flavonoid derived from the root of a medicinal herb, *Scutellaria baicalensis* Georgi)[73], and *Graptopetalum paraguayense* E Walther leaf extracts[74]. Pretreatment with these bioactive components diminishes microgliamediated inflammatory activity and neutrophil responses after brain ischemia.

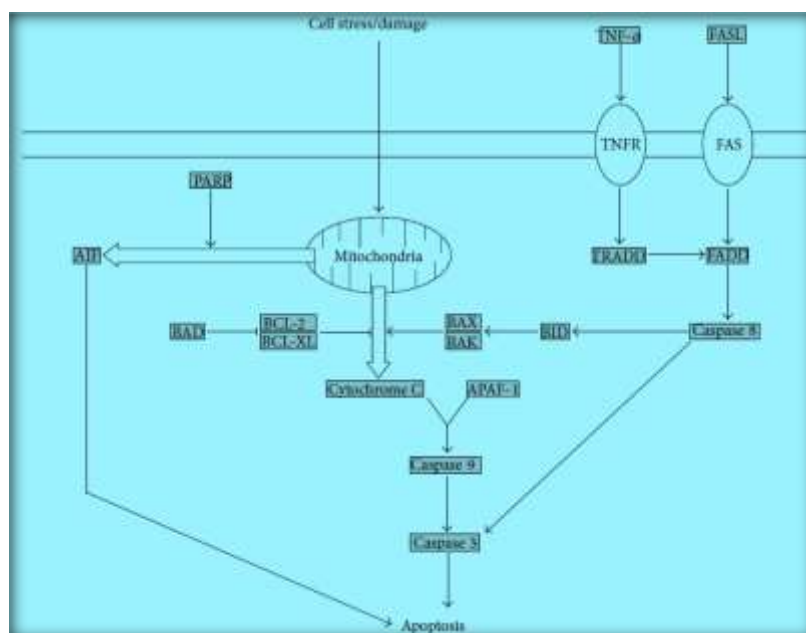


Figure 5. Mechanisms of induction of apoptosis. In the classical pathway, mitochondria release cytochrome C in response to cell stress and damage, leading to activation of caspase 9 and subsequent activation of caspase 3 and other effectors of apoptosis. Alternatively, mitochondria may also release apoptosis-inducing factor (AIF), which leads to apoptosis by a caspase independent mechanism. The death receptor pathway involves the activation of FADD by various cell signal receptors, followed by activation of caspase 8 and the subsequent caspase cascade leading to apoptosis.

Few studies have elucidated in detail the precise target for natural anti-inflammatory agents used to treat cerebral ischemia, suggesting that its precise mechanism of neuroprotection is still far from definite. Recently, several new targets closely related to inflammation, such as peroxisome proliferator- activated receptor- γ (PPAR γ) and NF- κ B, have been reported to mediate inflammation in activated macrophages by regulation of gene promoter regions, leading to inflammatory gene transcription [75]. Some plant-derived compounds exhibit direct regulation of PPAR γ and NF- κ B. For example, unique polychlorinated compounds named chlorophyllins A-C exhibit potent PPAR γ agonistic effects [76] and prenyloxycinnamic acid derivative 4'-geranyloxyferulic acid, obtained from *Acronychia baueri* Schott, also increases PPAR γ activity significantly [77]. Incensole acetate and its nonacetylated form, incensole (IN), isolated from *Boswellia* Resin, a major anti-inflammatory agent in the herbal medical tradition, inhibits NF- κ B activation [78]. The anti-inflammatory compound parthenolide from the medicinal herb Feverfew (*Tanacetum parthenium*) directly binds to and inhibits I- κ B kinase [79]. Hesperetin, a flavanone derived from citrus fruits, suppresses NF- κ B activation in both young and old rats through multiple signal transduction pathways [80]. These exciting studies indicate that nuclear transcription factors may serve as the direct target of natural anti-inflammatory compounds. More attention should be paid to the role of these transcription factors in the neuroprotective effects of natural anti-inflammatory compounds.

Apoptosis as a Target for Neuroprotection

During stroke, the diminished supply of oxygen and glucose to the brain leads to reduced cellular metabolism and depletion of energy stores. Combined with tissue damage due to mechanisms such as those mentioned above, cell death by either necrosis or apoptosis may be initiated. In the context of intervention, apoptosis is preferable to necrosis because it can be blocked by various treatments, allowing damaged tissue to be rescued. Cells within the core infarct typically die by necrosis, whereas those in the penumbra die by apoptosis. The primary factor in determining which mechanism of cell death occurs is the level of ATP within the cell [81]. ATP is required for the process of apoptosis, and cells with insufficient ATP stores will die by necrosis instead. Apoptosis can occur by several pathways, as shown in Figure 5.

The mitochondrial pathway can proceed through either caspase dependent or caspase independent mechanisms. Alternatively, apoptosis may be induced by the death receptor pathway. In the caspase dependent pathway of mitochondrial apoptosis, release of cytochrome C from mitochondria results in activation of caspase 3, which initiates a caspase cascade leading to the degradation of cellular components and cell death. Caspase 3 activity is commonly used as an indicator of apoptosis. Reduction of activated caspase 3 levels is therefore a goal of many neuroprotective treatments. Tanshinone IIA decreases the levels of cleaved caspase 3 in tMCAO rats, resulting in a reduction in infarct volume, edema, and neurological deficits. Diallyl sulfide reduces expression of caspase 3 and increases expression of BCL-2, an endogenous antiapoptotic protein, in tMCAO rats as well. Hypothermia has been shown to reduce caspase 3 levels for up to 1 week after focal cerebral ischemia in rats [82]. Pioglitazone, an agonist of the peroxisome proliferator-activated receptor γ (PPAR γ), activates STAT3 in tMCAO rats, leading to changes in the expression of antiapoptotic genes and reduced levels of caspase 3 [83]. Caspase 3 is not the only potential therapeutic target within this pathway, however. The cellular inhibitors of apoptosis (cIAPs) are endogenous molecules that bind to caspases and block their activation. Ischemic preconditioning has been shown to increase the levels of cIAP1 in neurons and reduce apoptosis following unilateral CCAO in rats [84].

Although apoptosis is most commonly associated with the caspase dependent mitochondrial pathway, other pathways also contribute to cell death after stroke, and neuroprotective agents that act upon these pathways are being investigated. The caspase independent pathway of apoptosis is characterized by mitochondrial release of apoptosis-inducing factor (AIF), which is stimulated by the activity of poly(ADPribose) polymerase (PARP). Several treatments that inhibit the caspase dependent pathway of apoptosis have also been shown to inhibit the caspase independent pathway as well. Ethanol administration decreases the expression of both caspase 3 and AIF up to 24 hours after tMCAO in rats [85]. The nitric oxide donor (S)-ZJM-289 suppresses the release of both cytochrome C and AIF from mitochondria and significantly reduces injury in MCAO rats [86]. Cyclosporin A has been shown to decrease the expression of caspase 3, AIF, and cytochrome C in a rat model of SAH [87].

Some compounds have been identified recently that target caspase independent pathway specifically. Ginsenoside-Rd has been shown to inhibit PARP-1 activity and AIF release in rats subjected to MCAO [88]. The death receptor pathway of apoptosis differs from the other two in that mitochondria are not required for its induction. Similar to the caspase dependent pathway of mitochondrial apoptosis, however, the death receptor pathway also uses caspase 3

as an effector. Treatments that affect the level and activity of caspase 3 may therefore block this pathway as well. Treatments that target this pathway directly have also been identified. Muscone decreases the expression of the death receptor FAS and reduces apoptosis in MCAO rats [89]. Antibodies against TNF α , another initiator of the death receptor pathway, block changes in the expression of caspase 3 after SAH in rats [90].

Natural compounds with anti-apoptotic effects

Ischemic cerebral injury is known to induce histopathological damage and related neurological deficits, leading to the activation of complex neurochemical cascades of cell death, which are primarily expressed as apoptosis. In principle, these apoptotic cascades are reversible and form an important aspect of the penumbra concept, a major target of therapeutic interventions. In general, ischemia and reperfusion induced neuronal apoptosis can be classified into two types: caspase-dependent and caspase-independent pathways. Caspases are intracellular proteases that function as initiators and effectors of apoptosis. When activated, caspases cleave a variety of intracellular proteins, including major structural elements in the cytoplasm and nucleus, components of the DNA repair machinery, and a number of protein kinases [91]. Thus, caspases may be effective against ischemia-induced neuronal cell apoptosis by blocking apoptotic cascades with appropriate drugs. An alkaloid-free ethyl acetate, extracted from the root of *Sophora flavescens*, has been reported to protect against focal cerebral ischemia by decreasing DNA fragmentation and inhibiting caspase-3 activity directly [92]. In that study, it was found that caspase-independent programmed cell death, mediated by the translocation of apoptosis-inducing factor (AIF) from the mitochondria to the nucleus, plays a key role in ischemia. During ischemia and reperfusion injury, in the case of massive and irreparable DNA damage, overactivation of Poly(ADP-ribose) Polymerase-1 (PARP-1) can lead to necrotic cell death caused by the depletion of NAD⁺ and ATP, as well as enhance AIF release from mitochondria [93]. A series of PARP-1 antagonists have been developed, some of which have become promising drug candidates [94-95]. However, the characterization of natural compounds regulating PARP-1 remains to be elucidated.

It is thought that berberine performs its anti-apoptotic effect by inhibiting both caspase-dependent and independent pathways [96]. Synergistic inhibition of caspase-dependent and independent neuronal cell apoptosis is more likely to be effective as a therapeutic approach for the treatment of ischemia. Numerous herb drugs have been demonstrated to act on anti-apoptotic pathways, such as Bcl-2 family proteins. The abundant expression of Bcl-xL protein in the adult brain is known to suppress activation of procaspase 9 by forming a complex with Apaf1 and to prevent the release of cytochrome c from mitochondria, thus maintaining cell viability. Therefore, Bcl-xL becomes a promising target for drug intervention to reduce cell apoptosis [97]. Pretreatment with 4-hydroxybenzyl alcohol 30 min before ischemia, one of the major active phenolic constituents of *Gastrodia elata* Blume, could antagonize cerebral ischemia by increasing Bcl-2 expression and inhibiting caspase-3 activity, leading to the amelioration of cell apoptosis in ischemic regions [98]. Direct evidence from preclinical research shows that Ginsenoside Rb1 (gRb1) regulates the anti-apoptosis signaling pathway, which stimulates the expression of mitochondrion-associated anti-apoptotic factor Bcl-xL through the use of reporter plasmids. The transcription factor signal transducer and activator of transcription 5 (Stat5) are known to activate Bcl-xL family proteins via binding to the bcl-xL promoter. The Stat5 responsive element in the bcl-xL promoter becomes active in

response to gRb1 treatment, suggesting that the Stat5 pathway participates in anti-apoptotic effects by regulating Bcl-2 indirectly through activation of gRb1 [99]. Molecular pharmacology research in this direction should elucidate the direct target of natural drugs that regulate apoptotic signaling pathways.

Excitotoxicity as Target for Neuroprotection

During stroke, depletion of neuronal oxygen and energy reserves leads to the release of toxic amounts of the neurotransmitter glutamate into the extracellular space [100-101]. The subsequent activation of glutamate receptors causes an influx of calcium and neuronal depolarization, resulting in aberrant activation of numerous calcium-dependent pathways in the brain and initiation of the processes of necrosis, apoptosis, and autophagy. Glutamate excitotoxicity therefore plays a significant role in the pathology of stroke, and a large number of research studies are devoted to suppressing its effects. One method of neuroprotection against excitotoxicity is to reduce the amount of glutamate release during stroke. The *Ginkgo biloba* extract EGb761 has been shown to significantly decrease striatal glutamate levels in mice subjected to MCAO, accompanied by reduced neurodegeneration and edema [102]. The individual constituents of EGb761 also have neuroprotective properties [103]. Another method of protection is to block the action of glutamate receptors in the brain. The microRNA miR-223 reduces expression of the glutamate receptor subunits GluR2 and NR2B, and is neuroprotective against transient global ischemia [104]. Activation of the transient receptor potential vanilloid 4 (TRPV4) is associated with increased activity of the NMDA receptor. HC-067047, an antagonist of TRPV4, reduces the extent of infarct after tMCAO in mice [105]. Magnesium sulfate is an antagonist of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor and in some studies has been shown to improve recovery in humans with acute ischemic stroke [106]. On the other hand, large scale clinical trials such as the intravenous magnesium efficacy in stroke (IMAGES) trial concluded that magnesium did not significantly improve outcome in ischemic stroke patients but may be beneficial in lacunar stroke [107]. It is possible that the timing of treatment is critical; therefore the field administration of stroke therapy-magnesium (FAST-MAG) trial was devised to test the benefits of magnesium given prior to arrival at a hospital.

Results from a pilot trial suggest that early administration of magnesium may have benefits, and phase III trials are currently underway [108]. Inhibiting the influx of calcium into cells following glutamate receptor activation can also be beneficial. Overexpression of the transient receptor potential canonical 6 (TRPC6) suppresses the increase in calcium induced by NMDA and reduces infarct size and mortality in mice [109]. Hyperforin, an activator of TRPC6, has also been found to be neuroprotective following tMCAO in rats [110]. The compound ginsenoside-Rd decreases expression of the calcium channel TRPM7 in MCAO rats, which may be partially responsible for its neuroprotective effects [111].

Natural compounds with calcium antagonization (Excitotoxicity)

ROS bursts and excitatory amino acid toxicity caused by ischemic reperfusion will lead to intracellular Ca^{2+} overload. Ca^{2+} overload in neurons is an essential signal of catastrophic events leading to irreversible neuronal injury. As a result, effects of herb components on Ca^{2+} overload induced by ischemic reperfusion have been studied extensively. Some herb components, for example, guattegaumerine (bisbenzylisoquinoline alkaloid from *Guatteria gaumeri*) and TSG, are found to reduce ischemia induced Ca^{2+} overload in neurons [112].

However, the exact mechanisms have not been elucidated in most studies. The main pathways of Ca^{2+} overload after cerebral ischemia and reperfusion are as follows: 1) the depletion of ATP leads to inactivation of Na^+, K^+ -ATPase, an enzyme regulating ionic concentration gradients for the generation of action potentials in neurons, with resultant depolarization of cell membrane potential and further opening of Ca^{2+} -permeable cation channels such as voltage-gated calcium channels (VGCCs)[113]; 2) the abundant release of excitatory amino acid activates α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, thereby further depolarizing the cell membrane potential and resulting in massive activation of NMDAR[114]; 3) the activation of NMDAR induces NADPH oxidase-or mitochondria pathway-dependent ROS generation, with subsequent Ca^{2+} release from intracellular calcium stores and influx through transient receptor potential melastatin (TRPM) channels, mediating ROS-dependant Ca^{2+} overload[115]; 4) extracellular acidulation could activate some acid-sensing ion channels and receptors, especially the Ca^{2+} permeable acid-sensing ion channel 1a (ASIC1a)[116]. Honokiol, a component of the herb *Magnolia officinalis*, can attenuate the decrease in Na^+, K^+ -ATPase activities induced by MCAO, but it has not been clarified whether the agent exhibits its effect by acting directly on Na^+, K^+ -ATPase or by attenuating the pathological inducer [117]. Use of VGCC blockers has been considered a therapeutic approach for post-stroke neuroprotection in humans for many years. Previous studies have shown that some isoquinoline alkaloids contained in medicinal herbs such as berberine (an alkaloid derived from the herbal medicine *Rhizoma coptidis* and palmatine (a flavonoid in propolis) exhibit significant and rapid inhibition of voltage-gated calcium currents in many native cells [118]. Interestingly, some of these alkaloids also exhibit neuroprotective effects against several central nervous system diseases [119].

There are few reports about the direct inhibition of NMDA receptor by natural products. Recently, some cation channels closely related to Ca^{2+} overload and selectively activated in certain pathological conditions have been a focus of drug development, including ASICs and TRPM. ASICs, especially ASIC1, which has been reported to be activated by extracellular acidosis, play a key role in ischemic cerebral injury [120]. In recent studies, puerarin, extract from *Radix puerariae*, has been reported to inhibit ASICs current in both natural cells and transfected cells, indicating the advantages of isolating novel ASIC antagonists with low toxicity from natural products [121]. Inhibition of TRPM, a Ca^{2+} -permeable nonselective cation channel, increases the resistance of neurons to ischemic death after brain ischemia and then preserves neuronal morphology and function. At present, several lines of evidence have demonstrated that accumulation of ADP-ribose induced by oxidative stress has led to the rapid opening of TRPM2 channels and calcium influx. TRPM2 might be the major pathway of cellular calcium overload induced by oxidation. Blockade of TRPM2 could reduce the cellular injury induced by oxidation. However, few research efforts have been conducted to explore compounds isolated from natural products that antagonize ADP-ribose or block TRPM2 channels [122-123].

Certain natural drugs are able to decrease the intracellular Ca^{2+} overload induced by oxidation. For example, salvianolic acid B, isolated from *Radix Salviae miltiorrhizae*, protects PC12 cells against hydrogen peroxide-induced intracellular calcium overload [124]. More interestingly, the structure-activity relationship of quercetin, which is abundant in various fruits and vegetables such as apple and carrot, as well as red wine and tea, has been revealed to antagonize hydrogen peroxide-induced calcium dysregulation in PC12 cells [125]. In one study, hydrogen peroxide induced an intracellular calcium elevation that could not recover the

Ca²⁺ levels in PC12 cells, but quercetin antagonized the effects of hydrogen peroxide in that cell model. Structure-activity relationships of five flavonoids were examined and the results indicate that two structural components, including (i) 3',4'-hydroxyl (OH) groups in the B ring and (ii) a 2,3-double bond in conjugation with a 4-oxo group in the C ring, as well as the polyphenolic structures, are crucial for the protective effect. Based on the significance of TRPM channels in calcium overload induced by oxidation, more attention should be paid to the natural compounds that antagonize hydrogen peroxide-induced calcium dysregulation by regulation of TRPM channels. These studies indicate that the natural products that can antagonize the ischemia-induced calcium overload might act on these novel targets involving ASICs and TRPM channels rather than on traditional targets such as L-type calcium channels and NMDA receptors. Nevertheless, more evidence and further studies are necessary to support this hypothesis.

Blood-Brain Barrier Disruption as a target for neuroprotection

Disruption of the bloodbrain barrier following stroke is commonly associated with the action of two matrix metalloproteinases, MMP-2 and MMP-9. MMP-2 is constitutively expressed at low levels in normal brain tissue; however stroke increases its expression and activity and also induces the expression of MMP-9. MMP-2 cleaves and activates MMP-9, which degrades components of the basement membrane in vascular walls and leads to BBB disruption. Other factors involved in BBB permeability after stroke include the extent of tight junction formation between endothelial cells and the effects of treatment with tissue plasminogen activator. The activity of MMPs is regulated endogenously by the tissue inhibitor of matrix metalloproteinase (TIMP), and treatments that stimulate it may be of significant benefit in protection against stroke-related brain damage. Inhibition of the expression and activity of MMPs by other means may be protective as well. Ethanol has been shown to inhibit the increase in MMP-2 and MMP-9 expression after tMCAO in rats and significantly reduces brain edema [126]. *Apocynum venetum* leaf extract (AVLE) also alleviates symptoms of BBB disruption in tMCAO rats by inhibiting the expression and activity of MMPs [127]. Hyperbaric oxygen treatment improved BBB function in a rat embolic stroke model through modulation of MMP-9 but displayed reduced effectiveness when administered in combination with tPA [128]. It may therefore be of limited benefit in those stroke patients who receive tPA.

Treatments that increase the formation of tight junctions may also provide neuroprotection following stroke. Doxycycline increases the expression of tight junction proteins in tMCAO rats and also inhibits MMPs [129]. Kruppel-like factor 2 (KLF2) protects against tMCAO in mice by regulation of the tight junction component occluding [130]. The c-Jun Nterminal kinase (JNK) inhibitor SP600125 restores vascular tight junctions and alleviates BBB disruption in a rat model of subarachnoid hemorrhage [131]. The GTPase RhoA is known to play an important role in the regulation of endothelial tight junctions, and inhibition of RhoA by fibroblast growth factor preserves BBB integrity in a mouse model of intracerebral hemorrhage [132]. Additional neuroprotective strategies include those that alleviate the negative effects of tPA. High density lipoproteins have been shown to reduce hemorrhagic transformation and improve BBB integrity following tPA treatment in experimental models [133]. Neurotrophic factors can also be used to stimulate repair mechanisms and restore BBB function. Pigment epithelium-derived factor (PEDF), for example, has been shown to have

beneficial effects on both BBB permeability and lesion volume after ischemiareperfusion in rats [134].

Disruption of the blood–brain barrier occurs after stroke. Therefore, protection of the blood–brain barrier has become an important target of stroke interventions in experimental therapeutics. Whether curcumin prevents cerebral ischemia/reperfusion injury by protecting blood–brain barrier integrity was investigated in one study [135]. A single injection of curcumin (1 or 2 mg/kg, i.v.) for 30 min after focal cerebral ischemia/reperfusion in rats significantly diminished infarct volume, improved neurological deficit, decreased mortality, and reduced the water content of the brain. Further experiments using cultured astrocytes found that curcumin significantly inhibited inducible nitric oxide synthase (iNOS) expression and NO_x (nitrites/nitrates contents) production induced by lipopolysaccharide (LPS)/tumor necrosis factor- α (TNF- α). Curcumin also prevented ONOO[–] donor SIN-1-induced cerebral capillary endothelial cell damage. On the basis of these observations, the authors concluded that curcumin can ameliorate cerebral ischemia/reperfusion injury by preventing ONOO[–]-mediated blood–brain barrier damage. Curcumin treatment also decreased malondialdehyde levels, cytochrome c, and cleaved caspase-3 expression and increased mitochondrial Bcl-2 expression. These authors concluded that the neuroprotective action of curcumin is exerted by antiapoptotic mechanisms. Similarly, curcumin was shown as a potent neuroprotective agent against stroke in a number of related studies [137–139]. Some other nutraceuticals with potential as antistroke agents include allicin, apigenin, kaempferol, quercetin and sulforaphane [140–144].

Benefits of Combination Therapy for Neuroprotection in Stroke

The pathology of stroke is complex, with multiple overlapping processes leading to either damage or protection. Although considerable neuroprotection can be gained by targeting just one of these processes, the potential benefit is even greater if multiple mechanisms of damage can be suppressed at the same time. Targeting the same pathway with more than one neuroprotective agent can also be beneficial. Consequently, combination therapy with more than one drug has proven to be effective in several experimental studies. The additional benefit may be small, additive, or even synergistic in some cases. Progesterone plus vitamin D hormone, for example, reduces infarct size and neurological deficits in tMCAO rats to a greater extent than either treatment alone [145]. The combination of the anti-inflammatory properties of atorvastatin and the antioxidant properties of probucol also produces increased neuroprotection in pMCAO rats [146]. Simvastatin combined with granulocyte colony-stimulating factor (G-CSF) reduced recovery time in a rat model of intracerebral hemorrhage and improved outcome [147]. Another strategy for combination therapy is the use of one drug to block the negative effects of another, such as tPA. Mild hypothermia, high-density lipoproteins, activated protein C analog, and fingolimod have all been shown to reduce the incidence of hemorrhagic transformation following administration of tPA in several animal models [148–150].

Neuroprotective Treatments with Pleiotropic Effects

Although combination therapy can produce additional benefits in some cases, not all treatments are compatible with each other. Some combinations may have antagonistic effects, producing less benefit than either treatment alone. In some cases, combination therapies may enhance damage. Furthermore, combination treatment requires significantly more testing to

determine safety and effectiveness than a single compound. There is therefore considerable interest in the study of treatments with beneficial effects on more than one mechanism of stroke-related damage. Some of these treatments have only recently been discovered (Table 1) and are still in the early stages of investigation. Others, however, have already been the subject of considerable study and show great promise for the treatment of stroke, as summarized below.

Minocycline. Minocycline is a broad spectrum antibiotic which, in addition to its antibacterial activity, also has anti-inflammatory and antiapoptotic effects [151]. Consequently, it has been shown to be protective in a number of diseases including stroke. Minocycline is one of the few neuroprotective agents in animal studies that has also been proven effective in human trials. In one recent trial, oral administration of minocycline resulted in significantly improved outcomes as long as three months after stroke [152]. Furthermore, animal studies indicate that additional benefits are to be gained by combining minocycline with other neuroprotective strategies. Minocycline reduces the risk of subsequent hemorrhage following administration of tissue plasminogen activator in diabetic rats subjected to focal embolic stroke [153]. It also reduces infarct size and suppresses several hallmarks of inflammation. Minocycline plus normobaric hyperoxia has a synergistic effect on the reduction in infarct volume following tMCAO in rats and has a positive effect on hemispheric swelling that was not seen with either treatment alone [154]. This combination also resulted in greater inhibition of apoptosis and MMP activation. Minocycline improves recovery after transplantation of bone marrow mononuclear cells into ischemic rats, presumably by inhibition of microglial activation [155-156]. It also preconditions neural stem cells against oxidative stress, producing reduced infarct size and improved neurological performance following transplantation in rats exposed to tMCAO [157].

Carnosine. Carnosine is a naturally occurring dipeptide that has both antioxidant and antiexcitotoxic properties [158-159]. It also is an efficient chelator of metal ions such as zinc, which is required for the activity of matrix metalloproteinases. Preclinical studies have shown that carnosine is well tolerated and produces robust neuroprotection in animal models of both transient and permanent ischemia [160]. It also readily crosses the intact blood-brain barrier, which allows it to be administered even in the early stages of stroke. Carnosine reduces glutamate excitotoxicity in pMCAO mice, resulting in reduced infarct size and improved neurologic function [161]. In another study, carnosine was shown to decrease infarct size, MMP activity, and levels of reactive oxygen species [162]. Carnosine can also be cleaved by carnosinase into the amino acids alanine and histidine, which are neuroprotective as well. Bestatin, an inhibitor of carnosinase, increases damage in pMCAO mice [163].

Asiatic Acid. Asiatic acid is a plant-derived compound with effects on oxidative stress, inflammation, and excitotoxicity that has been shown to be beneficial in the treatment of wound healing, beta-amyloid toxicity, and liver injury. Recent evidence suggests that it may be neuroprotective in stroke as well. In pMCAO mice, asiatic acid reduces infarct size and improves neurologic scores, possibly by suppression of mitochondrial damage and BBB disruption [164]. Subsequently, asiatic acid was also found to be neuroprotective in multiple models of ischemia in rats by inhibiting mitochondrial damage and MMP-9 activation [165]. Asiatic acid also blocks the negative effects of excitotoxicity in mice following exposure to glutamate [166]. An extract of *Centella asiatica* has been shown to improve several markers of behavioral function and improved the antioxidant status in tMCAO rats [167]. This extract

contains asiatic acid as well as several other related compounds that also have neuroprotective properties. Further investigation of this class of molecules is therefore warranted to determine the extent of their effects.

Cannabinoids. Another major class of molecules with multiple beneficial effects in stroke are the cannabinoids. These compounds are primarily known for their anti-inflammatory effects in many diseases including stroke [168]. Recently, however, evidence suggests that cannabinoids may also have antioxidant and antiapoptotic effects [169]. The cannabinoid receptor agonists WIN55,212-2 and JWH-133 reduce activation of microglia and macrophages after induction of ischemia in mice and rats, resulting in reduced infarct size and neurological impairment, as well as protection of oligodendrocyte precursor cells [170–171]. Another receptor agonist, TAK-937, provides neuroprotection in tMCAO rats, and the neuroprotective effect is increased when given in combination with hypothermia [172]. Furthermore, TAK-937 not only is effective in rodent models of stroke but also has been shown to reduce infarct volume in nonhuman primates [173]. This specific compound is therefore of considerable interest for future use in human trials.

Flavonoids. In some cases, whole classes of molecules are known for having multiple neuroprotective effects. One such group of compounds that are currently the subject of intensive research are the flavonoids. These molecules are naturally occurring compounds that readily cross the blood-brain barrier and are well known for their protective effects. The flavonoids in cocoa, for example, have antioxidant properties and also promote perfusion, angiogenesis, and neurogenesis in the brain [174]. Xanthohumol has been found to have anti-inflammatory, anti-apoptotic, antioxidant, and antithrombotic properties. Following MCAO in rats, xanthohumol decreases the levels of TNF α , hypoxia-inducible factor 1 α (HIF-1 α), and inducible nitric oxide synthase (iNOS) [175]. It also reduces expression of activated caspase 3, scavenges hydroxyl radicals, and inhibits platelet aggregation. Treatment with naringenin results in neuroprotection in tMCAO rats through both antioxidant and anti-inflammatory mechanisms [176]. Galangin improves cerebral blood flow, inhibits apoptosis, and protects mitochondrial function after MCAO in rats [177]. In tMCAO mice, fisetin protects the brain against ischemic injury by suppressing activation of cerebral inflammatory cells and inhibiting the migration of macrophages and dendritic cells into the brain [178]. In models of intracerebral hemorrhage, baicalin has been found to attenuate edema of the brain and inhibit apoptosis [179].

Neuroprotective Agents in Human Clinical Stroke Trials

Perhaps the greatest challenge in the study of neuroprotection in stroke is the translation of animal studies to humans. Numerous treatments that produce robust protection in rodents have failed to provide significant benefit in clinical trials. The various theories on the reason for this failure have already been discussed elsewhere and will not be covered here [180–181]. Amid the abundance of discouraging results, however, a small number of neuroprotective strategies have shown promise in human stroke patients. A brief summary of recently completed clinical trials for the study of neuroprotection in ischemic stroke and subarachnoid hemorrhage is provided below.

Citicoline Trial on Acute Stroke (ICTUS)

Citicoline is a nutritional supplement that not only is commonly used to improve memory retention but also has been shown to prevent neuronal degeneration and improve visual

function. It has already been approved in some countries for the treatment of acute ischemic stroke. A randomized, placebo controlled trial was conducted to evaluate the efficacy of citicoline in patients with moderate to severe acute ischemic stroke [182]. A total of 2298 patients were administered either citicoline (1000 mg every 12 hours) or placebo for up to 6 weeks. Outcome was determined at 90 days based on the National Institute of Health Stroke Scale (NIHSS), modified Rankin Scale (mRS), and modified Barthel Index (mBI scores), plus the occurrence of intracranial hemorrhage, neurologic deterioration, or death. No significant difference in recovery was observed between the citicoline and placebo treatment groups.

Ginsenoside-Rd. Ginsenoside-Rd is a calcium channel antagonist that has been previously shown to be neuroprotective in human trials. An extended trial of ginsenoside-Rd was performed in 390 patients with acute ischemic stroke [183]. Subjects were administered ginsenoside-Rd or placebo intravenously over a 14-day period and evaluated using NIHSS and mRS scores for 90 days. Significant improvement was seen with ginsenoside-Rd in NIHSS scores at 15 days and mRS scores at 90 days.

Cerebrolysin. Cerebrolysin is a mixture of peptide fragments that mimics the action of neurotrophic factors and has been shown to be neuroprotective in a number of conditions such as hyperthermia-induced neurotoxicity, vascular dementia, Alzheimer's disease, traumatic brain injury, and stroke. A large, double-blind clinical trial was conducted to test the efficacy and safety of Cerebrolysin in patients with acute ischemic stroke [184]. A total of 1070 patients were administered aspirin and either Cerebrolysin (30 mL/day) or placebo over a period of 10 days. Although no significant difference between treatment groups was seen after 90 days, a positive trend was seen in those patients with an NIHSS score greater than 12.

Minocycline. Minocycline is an oral antibiotic with proven safety over years of use. In addition to its antibiotic properties, minocycline also has anti-inflammatory and antiapoptotic effects that have been shown to be neuroprotective in animal models of stroke and in previous human trials. The efficacy of oral minocycline was examined in a recent single-blinded open-label study [185]. Fifty patients with acute ischemic stroke were given either minocycline (200mg/day) or placebo for five days and assessed for various indicators of outcome at 1, 7, 30, and 90 days. Patients receiving minocycline showed significant improvement after 30 days in NIHSS, mBI, and mRS scores. NIHSS scores continued to be significantly improved at 90 days. Larger phase II and phase III trials are awaited.

Summary

The pathology of stroke is incredibly complex, and treatment of its devastating effects is a continuing medical challenge. The topic of neuroprotection in stroke is equally complex, as can be seen by the wide variety of approaches currently being studied by the scientific community. On the one hand, no treatment or combination of treatments can be expected to encompass the entirety of damaging processes that occur during stroke. In this respect, the search for better therapies is never ending. On other hand, the availability of a large number of neuroprotective strategies increases the probability that one or more will ultimately prove to be effective. This fact is particularly relevant considering that the largemajority of neuroprotective treatments developed in animal models have failed to produce significant benefits in human trials. As a result, treatment options for stroke are still limited. A few neuroprotective agents have shown promise, however, and it is hopeful that they may be approved for general use in the future.

The failure of preclinical studies to translate into clinical trials highlights the importance that these studies be properly designed. To this end, the Stroke Therapy Academic Industry Roundtable (STAIR) has developed a set of recommendations for the preclinical assessment of neuroprotective treatments [186]. These include consideration of the proper animal model, dosage level, and time points to be used, as well as the use of physiological monitoring and more than one measure of outcome. Recommendations for phase I/II clinical trials of potential stroke therapies have also been developed to facilitate the transition to phase III trials [187]. It is critical that both preclinical studies and clinical trials be designed to complement one another, in order to ensure that the results are comparable and to allow subsequent investigation of the reasons behind the success or failure of neuroprotective treatments in humans. It has also been proposed that, rather than proceeding directly from animal studies to clinical trials, international multicenter preclinical studies should be performed on promising neuroprotective agents to identify potential problems in translating from animals to humans [188-189].

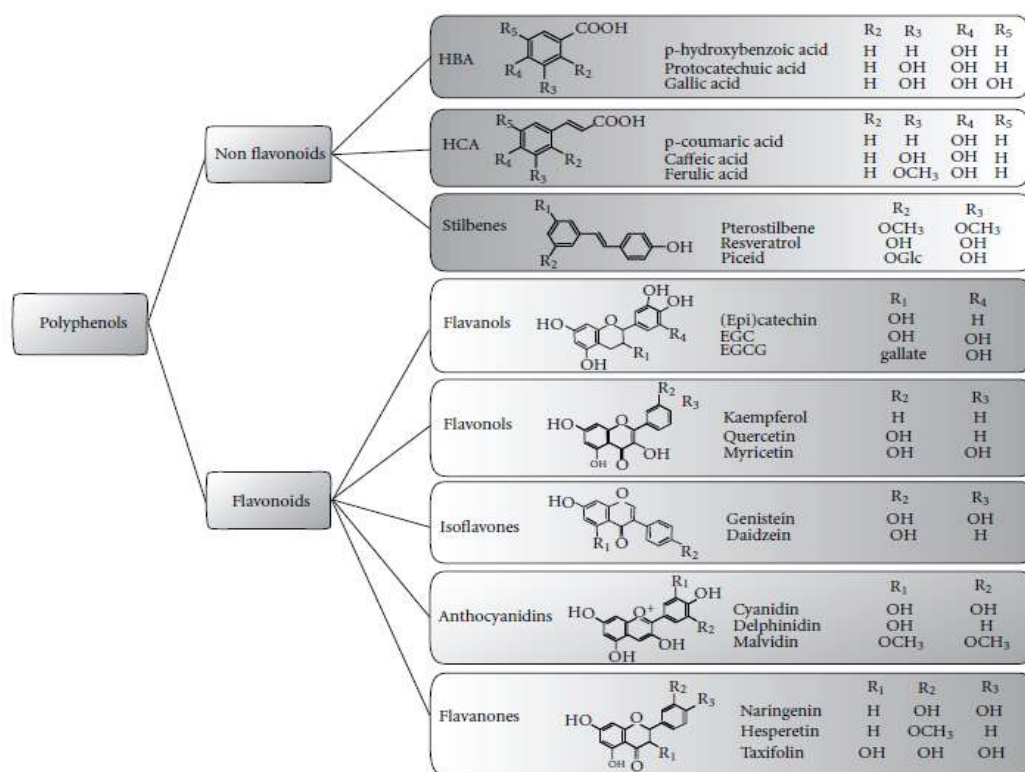


Figure 6. Structures of polyphenols. Polyphenols are a group of naturally occurring phytochemicals which are present in high amounts in fruits, vegetables, and natural products and are characterised by the presence of multiple hydroxyl groups on aromatic rings. These compounds are divided into two main categories, the flavonoids and non flavonoids, based on the number of phenol rings and the way in which these rings interact. For the flavonoid group, the major differences between the individual groups arise from the hydroxylation pattern of the ring-structure, the degree of saturation of the C-ring, and the substitution of the 3-position. HBAs, hydroxybenzoic acids; HCAs, hydroxycinnamic acids.

One complicating factor in the development of neuroprotective strategies is the dual nature of many of the processes that occur in the brain during stroke. The activity of MMPs, microglia, and other inflammatory cells, for example, can be either damaging or protective depending on the magnitude, location, and timing of their effects. Even mechanisms of cell death such as apoptosis and autophagy can be beneficial in the right circumstances. The development of potential neuroprotective treatments, therefore, must take both the positive and negative aspects of the stroke response into consideration, to ensure that they are administered under the conditions that are most appropriate and that will produce the greatest benefit.

POLYPHENOLS IN NEUROPROTECTION

Phenolics

Phenolics are ubiquitously found across the plant kingdom, with ~10,000 structures identified to date. Phenolics range from simple low-molecular weight compounds, such as the simple phenylpropanoids, coumarins, and benzoic acid derivatives, to more complex structures such as flavanoids, stilbenes, and tannins. Of these, the flavonoids represent the largest, most diverse group, encompassing some 6000 compounds, all of which share a common underlying structure of two 6-carbon rings, with a 3-carbon bridge, which usually forms a 3rd ring. Flavanoids can then be subdivided according to modifications of this basic skeleton into chalcones, flavones, flavonols, flavanones, isoflavones, flavan-3-ols, and anthocyanins [190].

A wide range of phenolic compounds in the CNS function, directly interact with neurotransmitter systems. As an example, in animal models, a diverse range of individual and combined flavonoids that occur in traditional medicinal extracts exert sedative/ anxiolytic effects via direct binding to GABAA receptors [191-192], cognitive enhancement via antagonistic GABAA receptor binding and resultant cholinergic upregulation [193], and antidepressant effects via monoamine oxidase inhibition and resultant increases in levels of 5-HT, DA, and noradrenaline in select brain areas [194]. In mammals and other vertebrates, phytoestrogens modulate hormonal systems, and therefore brain function, via a variety of mechanisms [195].

Polyphenols

Polyphenols are found in most plant-derived foods and beverages. There are over 8000 polyphenolic structures identified in plants. Polyphenols add to the sensory and nutritional qualities of plant foods. Polyphenols are often involved in the plant's defensive response against different types of stress such as ultraviolet radiation, pathogens, and physical damage. Because plants usually produce these polyphenols as a defensive mechanism, environmental conditions such as soil type, sun exposure, and rainfall along with other factors such as genetics factors, germination, degree of ripeness, processing and storage, and species variety can have effect on the polyphenol concentration. All polyphenols contain an aromatic ring

with one or more hydroxyl group. Most also have at least one sugar residues (glycosides) attached to the hydroxyl groups. They are classified into different groups depending on the number of phenol rings and chemical groups bound to the rings [196-198].

Polyphenols are a group of naturally occurring phytochemicals which are present in high amounts in fruits, vegetables, and natural products and are characterised by the presence of multiple hydroxyl groups on aromatic rings. These compounds are divided into two main categories: the flavonoids and nonflavonoids, based on the number of phenol rings and the way in which these rings interact (Figure 6).

Polyphenols contain a wide range of molecule sizes. Polyphenols, such as phenolic acids, are simple compounds, whereas the tannins are highly polymerized molecules. Flavonoids make up most of the polyphenols and they form the most important single group of polyphenols [196]. Table 1 summarizes the main classes of polyphenols, some representative phenolics in the groups, and their dietary sources. Polyphenols are usually recognized for their antioxidant capabilities. Phenolic antioxidants have been shown to inhibit the oxidation of lipids and other molecules and protect against free radicals [196, 199]. Polyphenols can react with radicals to form polyphenol radicals. The polyphenol radical is more stable and less reactive because of the ability of the phenol group to absorb extra electrons. Most polyphenols are conjugated by methylation, sulfation, or glucuronidation during metabolism. The antioxidant capability could be determined by the type of conjugate and its location on the polyphenol structure. This might be why certain polyphenols are better at scavenging superoxides, whereas others can scavenge the highly reactive oxygen-derived radical peroxynitrite. Their antioxidant capacity may also correlate with their ability to chelate metals. Specific polyphenols can chelate iron and possibly prevent the formation of free radicals by iron [200-203].

Table 1. Major Subclasses of polyphenols, Compounds, and Food Sources

Polyphenol Group	Name of Compound	Sources of the Polyphenol compounds
Flavonoids		
Flavonols	Quercetin, myricetin, kaempferol	Onions, apples, kale, red wine, green and black tea, broccoli, berries
Flavanones	Hesperetin, naringenin, eriodictyol	Citrus fruit, tomatoes, mint
Flavanols (Proanthocyanidins and catechins)	Epicatechin, epigallocatechin, epicatechin 3-gallate, epigallocatechin 3-gallate	Apricots, green and black tea, red wine, chocolate
Anthocyanins	Cyanidin, pelargonidin, malvidin	Strawberries, other berries, grapes, wine, tea, eggplant, cabbage
Isoflavonoids (phytoestrogens)	Genistein, daidzein, glycitein	Lentils, chickpeas, alfalfa, clover, flaxseed, soybeans
Flavones	Apigenin, luteolin, diosmetin, tangeretin, xanthetin, xanthosetin, wogonin	Parsley, celery, skin of citrus fruit
Phenolic Acids		
Benzoic acid derivative	Galllic acid, vanillic, syringic, hydroxybenzoic	Tea, strawberries, raspberries, blackberries, black radish, onions
Cinnamic acid derivative	p-Coumaric, caffeic, ferulic, sinapic acids, vanillin, syringaldehyde, p-hydroxybenzaldehyde	Blueberries, kiwis, plums, cherries, apples, cereal grains, coffee
Lignan	Secoisolariciresinol	Linseed, lentils, cereals, garlic, asparagus, carrots, pears, prunes
Stilbene	Resveratrol	Grapes, red wine
Saponin	Ginsenoside	Ginseng root
Other polyphenols	Curcumin	Turmeric

Polyphenols have been shown to have several other actions in addition to their antioxidant ability. Evidence show that they can inhibit the activities of several enzymes, including lipoxygenase, cyclo-oxygenase, xanthine oxidase, phospholipase A2, ATPases, aldole reductase, phosphodiesterases, topoisomerase I and II, protein kinase C, phosphoinositide 3-kinase, Akt/PKB, (protein kinase B) and mitogen-activated protein (MAPKs) kinases (MAPs) [200, 204-205]. Some polyphenols have weak estrogenic properties and others can inhibit the enzymes involved in estrogen metabolism, aromatase, and 17 β -hydroxysteroid oxidoreductase [205]. The reduction of several diseases has been linked to polyphenols. Cardioprotection and a reduction in certain types of cancer have correlated with consumption of phenolic antioxidants [196, 205]. There is also evidence for polyphenols to be antiallergic, antiviral, antibiotic, antidiarrheal, antiulcer, and anti-inflammatory agents. Polyphenols have been used to treat hypertension, vascular fragility, allergies, and hypercholesterolemia [196, 198-199]. Polyphenols have also been implicated in the prevention of neurodegenerative diseases.

Polyphenols protect neurons against oxidative stress thought to be one of the main causes of neurodegenerative diseases. Even a 10-fold higher concentration of ascorbate did not protect neurons similar to polyphenols [199]. Polyphenols attenuate ischemia-reperfusion injury by interfering with inducible nitric oxide synthase activity, inhibiting lipid peroxidation, decreasing the number of immobilized leukocytes during reperfusion, and reducing complement activation which results in a diminished inflammatory response [200]. Most importantly, in addition to their antioxidant actions, they also influence neuroprotective and neurorestorative signal transduction mechanisms [203]. Epidemiological studies show an inverse relationship between stroke and polyphenol consumption [197]. The dietary intake of polyphenols varies greatly among different societies. Isoflavone intake as a result of soy consumption ranges from 20 to 240 mg for Asians and from 1 to 9 mg in the United States and Western populations [198, 205].

The evidence presented in this section suggests the potential of polyphenols in both preventive and therapeutic usages for cerebral ischemia/reperfusion injuries. Furthermore, no toxic or other adverse side effects were reported with the dietary use of high concentration of polyphenols, although regulated clinical trials have not been performed. In addition, their bioavailability, absorption, and metabolism also require more studies, especially in humans. It would be particularly important to compare individual polyphenols with extracts of fruits, beverages, and vegetables in preclinical and clinical projects and to further investigate possible mechanisms of their effects. Numerous studies have indicated that compounds in an extract can act synergistically so it would be advantageous to use multiple polyphenols in the treatment of stroke. In particular, when stroke symptoms appear, a substantial damage has already taken place in the brain. Therefore, treatments have to start as early as possible in order to reduce further neurodegeneration and promote regeneration. However, the preventive use of plant products will be likely the most effective strategy for the treatments of stroke and other age-related neurodegenerative disorders.

Phenolics, and flavanoids in particular, are ubiquitous in plants and therefore represent an important component of a normal human diet. Epidemiological studies have suggested associations between consumption of phenolic-rich foods or beverages and various diseases, such as stroke, cardiovascular disease, and cancer [206] and neurologic disorders such as dementia/AD [207-208]. Naturally, multiple phenolic compounds coexist in foods. Many investigations utilizing animal models have demonstrated, for instance, that berry extracts

with high levels of anthocyanins or other polyphenols can reverse brain insult- and age-related cognitive decrements in rodents and that the actives can cross the blood brain barrier [209]. Similarly, in healthy humans, complex mixtures of cocoa-flavanols have been shown to increase peripheral vaso-dilation and cerebral blood flow during task performance, as indexed by functional MRI [210], and improve performance on cognitively demanding tasks [211]. However, the majority of the research in this area is concentrated on the effects of single molecules and the following includes a review of evidence surrounding the 3 most promising single molecule candidates. The chemical structure of curcumin, EGCG and resveratrol are shown in Figure 7.

Curcumin. Curcumin, a curcuminoid polyphenol responsible for the bright yellow color of the Indian spice turmeric (*Curcuma longa* L.), has been utilized for centuries within the Ayurvedic system of medicine for the treatment of a whole host of ailments, including inflammation [212]. Curcumin exerts varied and wide-ranging effects on molecular targets [213]. These include transcription factors such as NF- κ B, a master regulator of the antioxidant response; the protein kinases, which are involved with the majority of cellular pathways, especially those involved with signal transduction; enzymes such as heme oxygenase 1, a stress response protein whose expression is upregulated after curcumin consumption and associated with neuroprotection [214]; invasion and angiogenesis biomarkers such as matrix metalloproteinase 9, which are associated, among numerous other activities, with tissue repair; and inflammatory mediators such as NF- κ B and cytokines such as TNF α and IL-1 and IL-6 [215].

In animals, some of the physiological effects attributed to curcumin include activity against a range of neurologic diseases in animal models, including AD, multiple sclerosis, Parkinson's disease, age-associated neurodegeneration, schizophrenia and depression [216-221].

EGCG. A number of the catechin polyphenols that are abundant in tea (*Camellia sinensis* L.) are reputed to have pharmacologically active properties. The 4 main tea flavanols are: (-)-epigallocatechin, (-)-epicatechin, (-)-epicatechin-3-gallate, and EGCG, with EGCG generally thought to be the main and active component in green tea. The potentially neuroprotective effects of EGCG include direct effects seen in vitro in metal chelation [222], as an anti-inflammatory agent [223], and in the reduction of amyloid- β and amelioration of amyloid- β induced neurotoxicity [224-225], with these neuroprotectant properties being in part mediated via the activation of cell survival genes and modulation of protein-kinase c signaling [222]. EGCG has also been shown to facilitate cholinergic transmission [226], enhance neurite outgrowth [227], and modulate cerebral blood flow parameters in healthy humans. In vivo evidence from animal models suggests neuroprotective properties in the face of AD [228-230] and Parkinson's disease [231] and following ischemia/reperfusion injury [232-233]. Longterm administration of green tea catechins (63% EGCG) has also been shown to improve cognitive performance and increase antioxidant capacity in normal rats [234] and rats infused with amyloid- β [235]. EGCG was also found to significantly increase the lifespan of *Candida elegans*, although, interestingly, this was observed only during situations of increased heat and oxidative stress [236].

This might suggest that the life-extending (and perhaps other) effects of EGCG are due to antioxidant actions and an upregulation of stress resistance-related proteins such as heme oxygenase 1. Indeed, pretreatment of cells with EGCG is associated with an increase in levels of heme oxygenase 1 [237]. Despite the relatively small number of investigations into the

neuroprotective properties of EGCG in humans, epidemiological evidence reports that higher consumption of tea/green tea is associated with a reduced risk of neurodegenerative disorders [238] and a lower prevalence of cognitive impairment. Future research should therefore consider both acutely and chronically supplementing green tea catechins to young, healthy participants as well as to those with cognitive senescence.

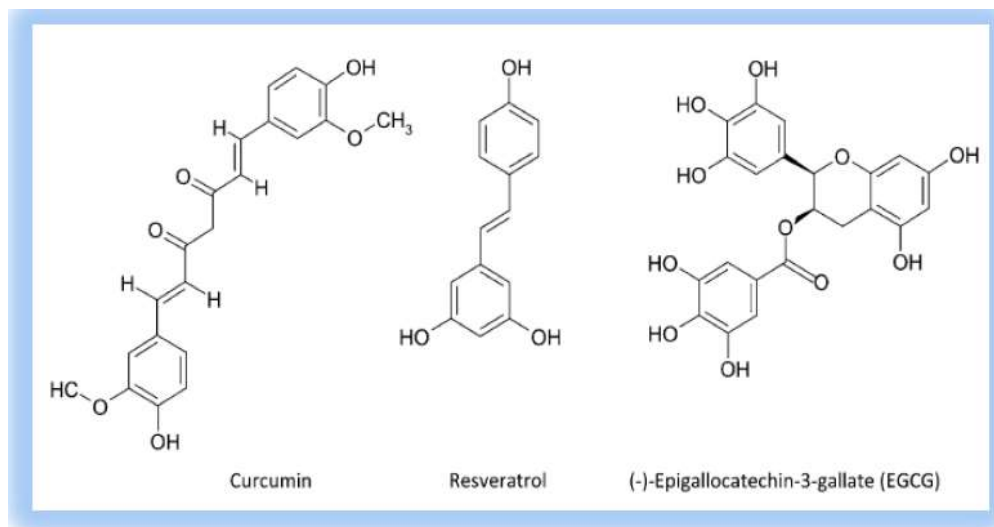


Figure 7. Structures of phenolic compounds like Curcumin, Resveratrol and EEG.

Resveratrol. The phytoalexin resveratrol (3, 4, 5 trihydroxystilbene) is produced within a range of edible plants in response to tissue damage and environmental stressors such as fungal and viral attack [239-240]. Consumption of resveratrol is associated with numerous protective health benefits in mammals, including increased longevity [241], antiinflammatory [242] and antiviral properties [243], and protection against cancer and tumorigenesis [244], cardiovascular disease [245], and atherosclerosis [246]. With regards to these latter 2 effects, resveratrol has been associated with the French paradox, whereby the consumption of red wine in some cultures has been suggested to contribute to a relatively low incidence of coronary heart disease despite a diet high in saturated fats [247-248]. Potential neuroprotective mechanisms of action include improving blood flow and perfusion [249] and the promotion of antioxidant defenses [250], which in vivo are likely to be as a result of resveratrol bolstering the bodies' own endogenous antioxidant defenses via upregulation of a host of antioxidant enzymes [251]. This may be partly a consequence of activation of the Nrf2 transcription factor, which plays a central role in the regulation of cellular redox status [252] and modulation of the protein kinases, which were observed to be involved with neuroprotection against amyloid- β -induced toxicity [253] in vitro [254] and in vivo, specifically in the hippocampus [255]. In vivo, oral administration has also been shown to diminish amyloid- β plaque formation in a region-specific manner in a transgenic mouse model [256].

Protective Role of Polyphenols in Neuronal Ischemic Injury

A significant interest on the protective effects of polyphenols has principally been because of their antioxidant properties. Phenolic antioxidants have been shown to inhibit the oxidation of lipids and other molecules and protect against free radicals [257]. Oxidative stress is a key event in the pathogenesis of cerebral ischemia. Overproduction of ROS during ischemia and/or ischemia/reperfusion can damage lipids, proteins, and nucleic acids, thereby inducing apoptosis or necrosis. Increasing evidence supports the hypothesis that plant polyphenols provide protection against neurodegenerative changes associated with cerebral ischemia [258]. Whether regional differences exist in the brain in the protective effects of polyphenols in ischemic injury is not clear. Most studies have reported the protective effects of polyphenols in the hippocampal and cerebral cortex regions in ischemia. Inanami et al. [259] observed a dose-dependent protection against hippocampal neuronal death in ischemia in gerbils after ad libitum oral administration of catechin in the drinking water for 2 weeks. Epigallocatechin-3-gallate (EGCG) also protected the hippocampal region in gerbils after transient global ischemia [260] and neuronal damage in a rat model of transient focal cerebral ischemia [261]. EGCG (50 mg/kg; intraperitoneal) was effective even when it was administered 3 hr after the ischemic insult in gerbils [262]. Hong et al. used green tea extract in the drinking water ad libitum for 3 weeks before ischemia in gerbils. This treatment reduced the infarct volume, the number of apoptotic cells, and lipid peroxidation, and inhibited the ischemia-induced hyperactivity [263]. In another focal ischemia model using middle cerebral artery occlusion (MCAO) in rats, the protective effects of resveratrol were shown with pretreatment for 21 days (20 mg/kg intraperitoneally per day). The treatment reduced the infarct volume, prevented motor impairment, and inhibited lipid peroxidation [264]. A single dose of resveratrol (20 mg/kg) given orally 1 hr before permanent middle cerebral artery ligation in mice did not protect against ischemic damage. However, when given daily for 3 days before ischemia, resveratrol significantly reduced the infarct size. In another study, effects of resveratrol on transient global cerebral ischemic injury were examined in gerbils [265]. Resveratrol (30 mg/kg given intraperitoneally per day) was injected either during or shortly after common carotid artery ligation and 24 hr later. Resveratrol significantly decreased neuronal death in the hippocampus and also inhibited glial cell activation. Nanocapsule encapsulated quercetin treatment resulted in significant protection to endogenous antioxidant enzymes against ischemia induced oxidative damage in neuronal cells of young and old rats [266]. Not many studies have reported the effects of polyphenols in the striatum except that by Shukla et al. [267] who saw a significant inhibition in lipid peroxidation and an increase in superoxide dismutase (SOD) activity in corpus striatum in rats pre-treated with curcumin prior to MCAO.

Some studies have examined the protective effects of polyphenols in the striatum but not in ischemic injury. For instance, GTE and EGCG were effective in preventing the depletion in striatal dopamine and tyrosine hydroxylase protein levels in a mouse model of Parkinson's disease [268]. It appears from these studies that protective effects of polyphenols would potentially be observed in the striatal region if assessed in ischemic injury. As mentioned above, cerebral cortex is another region where ischemic injury has been observed. Shukla et al. [267] reported an antioxidant effect of curcumin in the cortex of rats subjected to MCAO. Red wine polyphenol compounds also protected against oxidative stress in rats following MCAO/reperfusion [269]. Similarly, resveratrol significantly attenuated neuronal death in and decreased the generation of ROS, lipid peroxidation and nitric oxide (NO) content in the

cortex of rats subjected to transient global ischemia [270]. 2,3,5,4'-tetrahydroxystilbene- 2-O-beta-D-glucoside (TSG), an active component of the rhizome extract from *Polygonum multiflorum*, significantly reduced infarct volume in the cortex following MCAO [271]. Taken together, these studies indicate that polyphenols either exert or have the potential to exert neuroprotective effects in various regions in the brain that are vulnerable to ischemic injury. While the precise dose required to achieve a neuroprotective effect in cerebral ischemia is not clear and may vary with individual polyphenols,

Sutherland et al. [261] have reviewed the effects of green tea catechins including the safety and efficacy of such catechins. One mechanism underlying the neuroprotective effect of polyphenols is possibly through its effects on reducing the levels of apoptotic markers. Pomegranate polyphenols and resveratrol protect neonatal mouse brain from ischemic injury by reducing caspase-3 and calpain activation [272]. In neonatal rats, amentoflavone blocked the activation of caspase-3 and the proteolytic cleavage of its substrates following hypoxic-ischemic injury [273]. Pomegranate juice also diminished caspase-3 activation in the hippocampus and cortex of the neonatal brain against a hypoxic-ischemic insult through supplementation of the maternal diet with pomegranate juice [274]. Mangiferin and morin, two antioxidant polyphenols, are neuroprotective in both in vitro and in vivo models of ischemia possibly by reducing Ca^{2+} influx and decreasing caspase-3 [275]. A subsequent in vitro study by Campos-Esparza et al. [276] demonstrated that mangiferin and morin reduced the formation of ROS and restored the mitochondrial membrane potential following excitotoxic stress, which is a major component of ischemic injury. Further, these polyphenols also reduced the glutamate-induced activation of calpains, normalized the level of cytosolic Bax and inhibited the release of AIF from mitochondria. These actions of mangiferin and morin could well be part of their profile in an in vivo model of ischemic injury. EGCG, a green tea polyphenol, reduced up-regulation of MMP-9 activity and neuronal damage following transient focal cerebral ischemia in C57BL/6 mice [277]. MMP-9 downregulation by resveratrol was also observed in an in vitro model of neuronal ischemic injury [278]. 5,7,3',4',5'-pentahydroxy dihydroflavanol-3-O-(2"-Ogalloyl)-beta-d-glucopyranoside (AP1), a polyphenolic compound isolated from *Anogeissus pendula* Edgew (an arid forest tree), was effective in reducing apoptotic cells in rat brain following transient focal cerebral ischemia [279]. The effect of TSG in protecting rat brain from MCAO is by increasing the anti-apoptotic Bcl-2 proteins.

Curcumin, a potent polyphenol antioxidant enriched in turmeric, reduced cytochrome c release and subsequent caspase-3 activation following global cerebral ischemia in Mongolian gerbils [280]. While the aforementioned studies have demonstrated a decrease in caspase-3 levels in the presence of polyphenols, it is unclear whether polyphenols act directly on caspase-3 or whether they act on upstream caspases that are precursors to caspase-3. Alternatively, such polyphenols could also be activating inhibitor of apoptosis (IAP) which would then inhibit caspase-3 activation. In addition, effects of polyphenols may also involve protecting mitochondrial dysfunction in ischemic injury as seen in vitro [281]. Preventing the decline in mitochondrial membrane potential following ischemic injury may subsequently confer protection against apoptotic cell death. In addition, resveratrol can induce neuroprotection by increasing mitochondrial ATP synthesis efficiency in rat brain following ischemia [282]. While these studies highlight the potential neuroprotective mechanisms by which polyphenols attenuate cell death in ischemia, their antioxidant and anti-inflammatory

effects may also contribute to their ability to reduce cell swelling and/or brain edema which can be deleterious to neuronal and glial functioning.

Role of Polyphenols in Attenuating Oxidative Stress and Mitochondrial Dysfunction in Brain Edema and Cell Swelling

Oxidative stress is a key component of ischemic injury including cell swelling and brain edema and polyphenols, due to their antioxidant properties, would be postulated to attenuate such injury. Reports on the beneficial effects of polyphenols on brain edema in ischemia are scarce. Resveratrol has been reported to reduce brain edema in rats following MCAO [283]. Lee et al. has reported a protective effect of green tea polyphenol EGCG against neuronal damage and brain edema after unilateral cerebral ischemia in gerbils. AP1, a polyphenolic compound, also reduced brain edema in rats after transient focal ischemia [284]. Recently the protective effects of polyphenols from green tea as well as cinnamon on glial swelling in cultures following ischemia-like injury has been reported [285]. Myricetin and quercetin also attenuated cell swelling following oxygen-glucose deprivation in C6 cultures [286]. While in cell culture studies polyphenols reduced cell swelling, it is possible that the reduction in cell swelling was not due to the antioxidant effects of polyphenols.

An increase in intracellular calcium is a key feature of ischemic injury [287]. Further, an increase in $[Ca^{2+}]_i$ can induce cell swelling as demonstrated in lacticidosis-induced glial swelling [288] and in hypo-osmotic swelling in cultured astrocytes [289]. It has been demonstrated that quercetin and myricetin both attenuate OGD-induced increase in $[Ca^{2+}]_i$. Also, such blockade of the rise in $[Ca^{2+}]_i$ by blockers of the L-type calcium channel as well as modulation of $[Ca^{2+}]_i$ through BAPTA, a calcium chelator, reduces cell swelling in C6 glial cultures [290]. Other studies have also shown a decrease in $[Ca^{2+}]_i$ following administration of polyphenols. Quercetin attenuated the H_2O_2 -induced calcium dysregulation in PC12 cells [291]. Quercetin, catechin, and resveratrol also inhibited cardiac voltage gated sodium channel in rat cultured myocytes, but had no effect on the reverse mode NCX, the Na^+ / Ca^{2+} exchanger [292]. Apple condensed tannins inhibit the increase in intracellular free Ca^{2+} concentration in RBL-2H3 cells induced by antigen stimulation [293]. EGCG reduces the glutamate-induced $[Ca^{2+}]_i$ increase by attenuating ionotropic Ca^{2+} influx in PC12 cells [294]. Nevertheless, these studies indicate that polyphenols have the potential to modulate calcium channels that are involved in cell volume regulation, but their role in attenuating glial swelling/cytotoxic edema in ischemia needs to be further elucidated.

Mitochondrial dysfunction is an important characteristic of ischemia. The mitochondrial permeability transition (mPT) has been implicated as one mechanism, or at least part of the mechanistic pathway, for cell swelling in cultured astrocytes following ammonia toxicity or TBI as well as in brain sections in ischemia [295]. Despite these studies, the role of the mPT in cell swelling is not clear. Recently it has been demonstrated that the attenuation of cell swelling and the prevention of the decline in mitochondrial inner membrane potential ($\Delta\Psi_m$) by immunosuppressants, cyclosporin A (CsA), but not FK506, are consistent with the role of the mPT mediating such events. Similar to CsA, CPE, and green tea polyphenols, also significantly prevented OGD-induced cell swelling and the decline in $\Delta\Psi_m$ in C6 glioma indicating that one mechanism by which CPE and GTE exert their protective effects is possibly by blocking the mPT. Interestingly, quercetin significantly attenuated cell swelling in C6 glial cells following OGD but did not block the dissipation of the $\Delta\Psi_m$ [290] indicating that other factors, besides the mPT, mediate the development of cell swelling in ischemic

injury. It is also possible that preventing the induction of the mPT may be sufficient in some cases but may not be always necessary.

An increase in inflammatory markers has been associated with brain edema [296] and could potentially cause damage to the BBB [297]. A disruption of the BBB is observed in vasogenic brain edema. A key characteristic of polyphenols is their anti-inflammatory property [298] and anti-inflammatory effects of polyphenols have been reported in cerebral ischemia [299]. Inflammatory molecules can damage mitochondrial function. For instance, exposure of rat astrocyte cultures to interferon γ , in the presence or absence of LPS, can increase NO production which can subsequently damage the mitochondrial respiratory chain complex function. Studies that investigated the role of polyphenols on BBB function in ischemia are scarce except that reported by Zhang et al. [300] which examined the effects of green tea polyphenols on BBB permeability following MCAO in rats. They report a decrease in BBB permeability in the ischemic region in the presence of green tea and a concomitant decrease in levels of caveolin-1, a protein involved in BBB functioning and permeability. Wang et al. and Lee et al. report a reduction in water content in the brains of animals following ischemia with resveratrol and EGCG respectively, but it is not clear if the edema that was measured was of the vasogenic or cytotoxic type. Likewise AP1, a polyphenolic compound, also reduced brain edema in rats following MCAO but the type of edema assessed is not clear. A reduction in BBB damage and water content in the brain following cerebral ischemia in rats was reported with curcumin [301]. Curcumin also decreased brain edema in rats following MCAO [302] but as with some other studies the type of edema examined is not clear. In a rat thromboembolic stroke model, curcumin reduced brain edema [303] most likely of vasogenic type.

In addition, curcumin was reported to significantly lower oxidized proteins and interleukin-1 β , a pro-inflammatory cytokine, elevated in the brains of AD transgenic mice [304]. IL-1 receptor 1 (IL-1R1)-null mice when subjected to hypoxia-ischemia showed reduced cytotoxic and vasogenic edema when compared to wild-type mice [305]. Taken together it is conceivable that curcumin could attenuate vasogenic edema following ischemia. Further, anti-inflammatory properties of polyphenols have been reported in other stresses and this knowledge can be applied to vasogenic edema in ischemia. Polyphenols found in cinnamon also have anti-inflammatory effects in vitro [306]. A reduction in TNF- α , an inflammatory cytokine, has been reported for green tea polyphenols [307] as well as dried plum polyphenols [308] and TNF- α is one agent that increases endothelial permeability in vasogenic edema. Also, increases in intercellular adhesion molecule (ICAM-1) and myeloperoxidases in rodent lung injury are attenuated by green tea polyphenols [309]. In addition, anti-cyclooxygenase 2 effects of resveratrol [310], as well as anti-MMP9 effects of resveratrol [311] and other polyphenols have been demonstrated. The importance of inflammation in vasogenic edema, taken together with the anti-inflammatory effects of polyphenols, indicates that the polyphenols may play a protective role in reducing vasogenic brain edema in ischemia.

Oxidative stress and mitochondrial dysfunction are key features of cerebral ischemia that affect neuronal viability after ischemia. Edema can further aggravate neuronal injury by affecting cerebral perfusion. Currently, there are few remedial agents to effectively reduce neuronal death or brain edema not only in ischemia but also in other neural injuries including traumatic brain injury. The potential for the use of polyphenols in the preventing cell loss or damage and edema in cerebral ischemic injury is tremendous. However, the cellular and

molecular actions of polyphenols involved in neuroprotection have to be elucidated further. Given the large proportion of the population affected by stroke and traumatic brain injury, and with few strategies to effectively attenuate brain edema and associated neural damage, it is important to determine the potential beneficial effects of dietary polyphenols in the prevention and alleviation of such damaging effects.

Role of Polyphenols in Preventing Neuroinflammation

Although neuroinflammation plays a critical role in brain host defence, it also contributes to the underlying neuronal loss in neurodegenerative disorders and to damages associated with cerebral ischemia [312]. Neuroinflammation is “driven” by activated resident glial cells (astrocytes and microglia) which result in invasion of circulating immune cells and the production of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6), nitric oxide (NO), prostaglandin E₂, chemokines, and reactive oxygen species (ROS). Amongst the numerous factors released by activated glial cells, excessive NO• production has been reported to induce neuronal cell death by damaging the mitochondrial electron transport chain function in neurons [313] therefore resulting in neuronal ATP synthesis disruption and in increased generation of ROS [114]. Furthermore, NADPH oxidase activation, an important event in activated microglia-induced neurotoxicity, has also been suggested to mediate both superoxide (O₂•-) production and to release proinflammatory molecules such as TNF- α [314]. NO• produced in microglia or astrocytes may react with O₂•-, produced by NADPH oxidase to generate the neurotoxic peroxynitrite radical (ONOO-) [315]. ONOO- has been observed to inhibit mitochondrial respiration, induce caspase-dependent neuronal apoptosis, and to induce glutamate release resulting in excitotoxicity and neuronal death [316]. Additionally, glial cytokine production may also play a deleterious role in neurodegenerative diseases by binding to specific cell surface receptors expressed in neurons and activating apoptotic pathways.

There has been much interest in the development of new drugs capable of preventing neuroinflammatory-mediated brain injury. Emerging evidence suggests that dietary polyphenols may exert neuroprotective effects by suppressing the activation of microglia, which mediates inflammatory processes in the CNS. Although rather complex, the main anti-inflammatory properties of polyphenols include: (1) an inhibitory role on the release of cytokines, such as IL-1 β and TNF- α , from activated glia; (2) an inhibitory action against iNOS induction and subsequent nitric oxide production in response to glial activation; (3) an ability to inhibit the activation of NADPH oxidase and subsequent ROS generation in activated glia; (4) a capacity to downregulate the activity of proinflammatory transcription factors such as NF- κ B through their influences of a number of glial and neuronal signaling pathways, such as MAPK cascade (discussed in details below) [317-318].

For example, the commonly consumed flavonol quercetin has been reported to inhibit neuroinflammation by attenuating nitric oxide production and iNOS gene expression in microglia [319] and by preventing inflammatory cytokine production, thus preventing neuronal injury [320-321]. However, one of the major physiological metabolites of quercetin, quercetin-3_-sulfate, failed to demonstrate any anti-inflammatory action. Nevertheless, these studies have employed quercetin concentrations (10–50 μ M) much higher than of those found in plasma after ingestion.

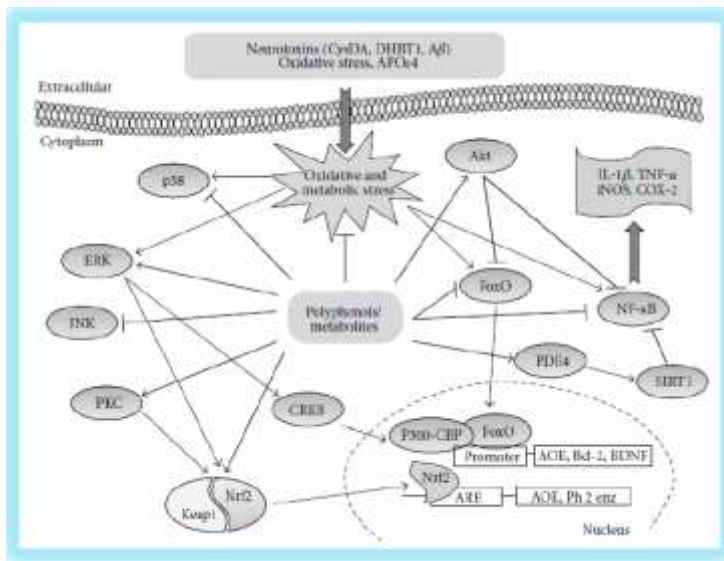


Figure 8. Mechanisms underlying the biological effects of polyphenols. Polyphenols and their *in vivo* metabolites activate cellular stressresponse pathways resulting in the upregulation of neuroprotective genes. For example, both PKC and ERK can activate the nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 then translocates to the nucleus and binds to the antioxidant response element (ARE) in genes that encode cytoprotective proteins such as antioxidant enzymes (AOE) and phase 2 (Ph2) enzymes. The transcription factor cAMP-responseelement-binding protein (CREB) is also activated by ERK, which induces the expression of brain-derived neurotrophic factor (BDNF), a mediator of neurohormesis. In addition, polyphenols can also regulate the transcription factor NF- κ B, which can mediate adaptive cellular stress responses by reducing the expression of inflammatory cytokines. Activated SIRT1 may also inhibit NF- κ B and so can reduce the cellular stress response. Another important pathway activated bymetabolic and oxidative stress involves transcription factors of the forkhead (FoxO) family, which modulate genes that encode antioxidant enzymes and other stress-response proteins.

In contrast to this, epicatechin and catechin (10–300 nM) were observed to inhibit TNF- α release but not iNOS expression or nitric oxide production in primary glial cells [322] suggesting that flavanols at physiologically relevant concentrations may hold the potential to exert anti-inflammatory effects in the central nervous system. Polyphenols present in blueberry have also been reported to inhibit NO $\cdot$, IL-1 β and TNF- α production in activatedmicroglia cells [323], and the flavanone naringenin was observed to be highly effective in reducing LPS/IFN- γ -induced glial cell activation [322].

Dietary polyphenols are also potent inhibitors of NADPH oxidase activity *in vitro*. A study comparing 45 polyphenolic compounds indicated that whilst both the flavanols (+)-catechin and (–)-epicatechin failed to inhibit NADPH oxidase, their relevant methylated metabolites exhibited strong NADPH oxidase inhibition through an apocynin-like mechanism [324]. Interestingly, other apocynin-like phenolic compounds, such as, ferulic acid, homovanillin alcohol, caffeic acid, tyrosol, and vanillic acid were also observed to inhibit NADPH oxidase activity, therefore indicating that smaller polyphenols, more structurally related to some colonic metabolites, may also serve as novel therapeutic agents in neuroinflammation (Figure 8).

There is also data which shows encouraging positive effects of polyphenols in animal and *in vitro* models relevant to multiple sclerosis (MS), a chronic debilitating disease which is characterised by demyelination, progressive irreversible axonal damage and inflammation [325]. For example, EGCG delivered orally reduces symptom severity in the autoimmune encephalomyelitis model of relapsing-remitting MS by reducing inflammation and increasing neuroprotection [326]. Quercetin has also been reported to be effective in the Experimental Autoimmune Encephalomyelitis (EAE) mouse model, and reduces T-cell proliferation *in vitro* at concentrations exceeding 10 μ M [327]. Micromolar concentrations of luteolin, apigenin, fisetin, and quercetin (but not morin or hesperetin) were reported to suppress the production of the cytokine interferon-gamma (IFN γ) from lymph-node-derived T cells but, paradoxically, worsen clinical severity in the EAE model. More recently, resveratrol protection against EAE was associated with rises in IL-17/IL-10 and with repressed macrophage IL-6 and IL-12/23 p40 expression [328]. Thus, the studies to date show promising proof of concept of beneficial effects of polyphenols in suppressing immune and inflammatory responses in models of MS.

Neuroprotection by Polyphenols in Hypoxic-Ischemic Injury in Neonates

Dietary supplementation with foods rich in polyphenols—pomegranates, blueberries, green tea, and apple juice—has been shown to provide neuroprotection in animal models of focal brain ischemia, of periventricular white matter injury, and of Alzheimer's disease [329-332]. Polyphenols have been found to possess antioxidant properties as well as to have effects on gene expression [333]. Specifically, one polyphenol, resveratrol, has been shown to increase activity of members of the sirtuin gene class, blunting p53 action and blocking apoptosis [334-335]. Recent studies indicate that among foods that contain polyphenols, juice extracted from the pomegranate has the highest concentration of measurable polyphenols [336-337]. The pharmacologic actions of pomegranate juice include antiatherosclerotic, antibacterial, and antiproliferative properties [338]. Recently it has been found that when the polyphenol rich pomegranate juice is consumed by the dam polyphenols from the juice are present in the pup and protected the pup against H-I brain injury [274]. Other studies have shown that the polyphenols caffeic acid phenylethyl ester and amentoflavone are also protective against neonatal H-I brain injury [273]. To test the hypothesis that it is the polyphenols of pomegranate juice that are responsible for neuroprotection, the effect of pomegranate polyphenol extract (PPE) in the neonatal H-I mouse model was tested and it was observed that supplementation of PPE to the drinking water of pregnant and nursing dams resulted in significantly decreased H-I induced caspase-3 activation. This suggests that it is the polyphenols of the pomegranate juice that are responsible for the neuroprotection.

Resveratrol the naturally occurring compound has been found to be neuroprotective in adult ischemia in rats when administered before the injury, but to our knowledge resveratrol has never been tested in neonatal H-I [339-341]. By examining a variety of different concentrations at several different time points it was found that IP injection of resveratrol leads to decreased caspase-3 activation in the P7 mouse in a concentration and time dependent manner. At doses of 200 μ g/kg or greater, resveratrol leads to decreased caspase-3 activation but only when resveratrol is injected prior to the injury. In addition to decreasing the caspase-3 activation resveratrol also decreases the calpain activation following neonatal

H-I, suggesting that it works as a generally neuroprotective agent and not just on the apoptotic pathway.

In addition to finding that resveratrol is protective in the neonatal mouse it was also demonstrated that resveratrol protects the neonatal rat against H-I induced caspase-3 activation. Although the injury paradigm is similar in rats and mice there are several neuroprotective agents that have been found to work only in one species. Since resveratrol has been found to protect against stroke in neonatal rats and mice as well as in adults, it could potentially be considered for further investigations in humans. Interestingly, the resveratrol was not found to be protective in the rat when given after the injury. Since the apoptotic cell death in the rat starts much later in the rat than in the mouse, and several drugs have been shown to be protective in the rat when given after the insult, we thought that resveratrol might follow the same pattern. The fact that resveratrol does not protect when given after the injury suggests that it is acting through proximal mechanisms in the cell death pathway initiated by H-I. One pathway that may be involved in the effects of polyphenols is via activation of the sirtuins such as SIRT1.

Polyphenols such as resveratrol may have beneficial effects on health via their antioxidant properties, suppression of inflammatory pathways, or other pathways such as activation of the sirtuin pathway [342]. Included in the sirtuin family is SIRT1, a human protein deacetylase that promotes cell survival by mechanisms such as negatively regulating the p53 tumor suppressor [343], deacetylating the transcription factor FOXO3 [344], repressing PPAR γ signaling [345] and modulation of NF- κ B dependent transcription [346]. Modulation of these pathways may provide a means to protect the developing brain against neonatal H-I induced brain damage. Recent studies show that polyphenols, including resveratrol, increase cell survival via activation of SIRT1 [41]. Parker et al. found that increased *sir2* gene dosage or treatment with resveratrol in *C. elegans* blocked neuronal dysfunction and cell death induced by polyglutamine expansion. Suggesting that resveratrol may act through a similar pathway in mammals, resveratrol protected mammalian neuronal cell lines from mutant huntingtin-induced cell death and this effect was inhibited by sirtuin inhibitors [347]. There is also evidence that resveratrol can block axonal degeneration via SIRT1 in the mammalian peripheral nervous system [348]. While increasing evidence suggests that resveratrol and other polyphenols are neuroprotective, whether their protective actions in the CNS *in vivo* are via SIRT1 has not been directly assessed. Determining the mechanism of protection of resveratrol, pomegranate polyphenols, and other polyphenols may lead to novel insights into both pathogenesis and treatment of neonatal H-I brain injury.

Resveratrol, a natural stilbene present at relatively high concentrations in grape skin and seeds and red wine, is known for its purported antioxidant activity in the vascular and nervous systems. In contrast to its direct antioxidant role within the central nervous system, recent research supports a protective mechanism through increasing endogenous cellular antioxidant defenses, which triggers a cascade of parallel neuroprotective pathways. A growing body of *in vitro* and *in vivo* evidence indicates that resveratrol acts through multiple pathways and reduces ischemic damage in vital organs, such as the heart and the brain, in various rodent models. Most of the protective biological actions of resveratrol have been associated with its antioxidative, anti-inflammatory, and antiapoptotic properties and other indirect pathways see figure 9.

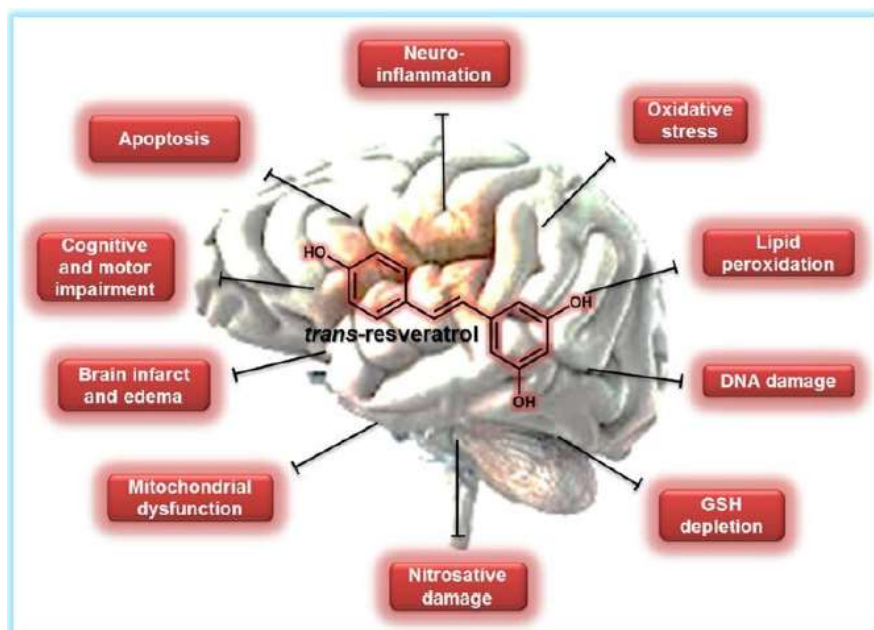


Figure 9. Potential targets associated with anti-stroke activity of resveratrol.

Resveratrol exhibits therapeutic response against stroke by preventing brain infarct, edema, mitochondrial dysfunction and cognitive and motor impairment. Furthermore, it diminishes nitrosative, oxidative, and DNA damage, which leads to preclusion of apoptosis and neuroinflammation

Summary

The neuroprotective actions of dietary polyphenols involve a number of effects within the brain, including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function. While many of the mechanisms underpinning their beneficial effects remain to be elucidated, it has become clear that they in part involve decreases in oxidative/inflammatory stress signaling increases in protective signaling, and may also involve hormetic effects to protect neurons against oxidative and inflammatory stressors. Most of the dietary polyphenols that have been shown to be protective against age-related disease are all chemically reactive and nearly all are electrophilic.

Such chemical features renders these molecules capable of influencing the redox potential of their target cells and to modulate series of transcriptions factors that result in the activation of phase I and phase II metabolism genes. Nonetheless, much of the data obtained on their bioactivity derived from short-term basis *in vitro* or *in vivo* studies where the dose used was not of nutritional relevance. Although at the moment, the balance of evidence that does suggest that polyphenol effects contribute to the benefits of a high intake of fruits and vegetables, the extent of their contribution *in vivo* and at physiological relevant concentrations remains uncertain. More work needs to be done to prove whether this class of compounds is most likely to result in health benefits and to determine their beneficial effects in slowly developing neurodegenerative disorders. In view of their multiple biological

activities, the consumption of polyphenol-rich foods throughout life holds a potential to limit neurodegeneration and to prevent or reverse age-dependent deteriorations in cognitive performance. However, the therapeutic and pharmacological potential of these natural compounds still remains to be translated in humans in clinical conditions. Moreover, efficacy in RCT is also needed to support the relatively consistent epidemiological and mechanistic evidence. Despite this lack of efficacy data and the uncertainty of their effects *in vivo*, investigations into the absorption and metabolism of various polyphenols in humans indicate that there are common pathways for the metabolism of the majority of polyphenols, notably via their bacterial metabolism in the large intestine. Consequently, research on developing dietary polyphenols for applications in neurodegenerative disorders should prioritise investigations of smaller polar polyphenols for brain bioavailability and bioactivity. The challenge ahead therefore is to proceed cautiously until rigorous randomized controlled clinical trials have been undertaken to determine empirically whether polyphenols and/or their metabolites have efficacy in individuals affected by dementia and other neurodegenerative conditions.

In general, the literature on the efficacy of the herbal extracts and phytochemicals reviewed here in terms of improving aspects of human brain function is somewhat equivocal. Research into the 2 alkaloids, caffeine and nicotine, is confounded by withdrawal effects and most of the remaining treatments have failed to progress beyond relatively small scale human studies. Indeed, in the case of the single molecule polyphenols (curcumin, resveratrol, EGCG), their huge and exponentially expanding literatures are singularly lacking in reports of relevant human intervention trials. Of the 3 treatments that have progressed to larger scale controlled trials and eventual meta-analyses, both GB and valerian are be devilled by methodological inconsistencies and inadequacies that make conclusions difficult to draw, with only St. John's Wort consistently demonstrating efficacy.

One consistent feature across the phytochemical groups is a gradation seen in terms of ecological roles and toxicity. Although something of a generalization, it is possible to characterize alkaloids as occupying the toxic extreme in terms of their deterrent effects in insects and other herbivores, with terpenes inhabiting the middle ground with a more mixed toxicity profile and a wider range of deterrent/ attractant/protective ecological roles. Phenolics then occupy the more benign end of the spectrum, exerting many internal protective roles and managing nontoxic interactions with herbivores and symbiotes. The same gradation could be suggested for the factors underlying the CNS effects in humans. Many of the behavioral effects of low doses of alkaloids are evidently the consequence of modulation of the same CNS mechanisms in both insects and humans and they elicit similar behavioral profiles given the comparative complexity of the taxa (Table 1). Although little research has addressed the effects of terpenes and phenolic compounds on insect behavior, it is possible to speculate that the CNS effects of terpenoids may be balanced between those predicated on similarities between human and invertebrate herbivores, e.g., the cholinesterase inhibitory and direct cholinergic and GABAergic receptor binding properties of many terpenes, and also the similarities between human and plant molecular physiology.

The phenolic compounds, particularly those like flavonoids that are ubiquitously consumed in plant-based foods, may then owe the balance of their CNS effects to the latter (but with notable exceptions in terms of hormonal effects and GABAergic effects). As well as the natural compatibility of molecules created by conserved stress signaling pathways common to both plants and humans, it is interesting to note that the induced

antibacterial/fungal and viral effects of curcumin, EGCG, and resveratrol within the plant may be mirrored by a similar protection conferred after exposure to similar pathogens in human cells and animal models. Although an exact concurrence between the mechanisms of action across the taxa has not yet been established, Friedman has demonstrated that, *in vitro*, the antibacterial, antitoxin, antiviral, and antifungal properties of tea flavonoids were similar against all of the food-borne pathogens reviewed.

These mechanisms ostensibly involved either binding to the invader and inactivating it or perturbing the membrane structure of the pathogen and causing leakage, with both resulting in preventing or limiting the deleterious effects of the bacteria, toxin, or virus. With phenolic compounds in particular it is also interesting to note that humans are likely to have lost the ability to synthesize vitamins, which include several terpenoids and methylated phenols, because the ubiquity of these micronutrients in our diet made it more advantageous in evolutionary terms to sequester them from food rather than synthesize them *de novo*. The same argument has been made for all dietary antioxidants, including many nonvitamin phytochemicals, and this proposition could be extended to include the nonantioxidant properties of groups of phytochemicals that occurred as part of our natural ancestral diet. This would largely accommodate the phenolic compounds, and flavonoids in particular, that are ubiquitous in plant foods. It may be relevant that most phenolic compounds have low parent molecule bioavailability but still exhibit *in vivo* bioactive effects. The rapid process of metabolism that takes place in the body could be viewed as the body processing the molecules into, for instance, glucuronidated and sulfated metabolites to more effectively transport and utilize them, in much the same way that vitamins are processed into their active metabolites and derivatives following consumption.

The gradation in toxicity and ecological/CNS functions is also seen in the comparative levels of research attention paid to the chemical groups. The alkaloid group has benefitted from intense research for over 200 yrs and has provided a multitude of medicinal compounds with CNS activity. Interest in terpenes, on the other hand, has really only escalated in the last 25 yrs, during which time many advances have been made in terms of characterizing the constituents and activities of complex plant extracts that often have low toxicity, high bioavailability, and a multitude of potentially relevant physiological effects. Similarly, research into the health effects of phenolic compounds has only reached any considerable level within only the last 15 yrs. In the case of alkaloids, they have proven particularly amenable to research and drug discovery because of their comparatively straightforward, single molecule modes of action. Evidence suggests that extracts with largely terpene or phenolic actives owe their effects to multifarious synergies between their component chemicals and this factor, along with an inability to reliably standardize extract constituents, has to date constrained their development and the clarity of the literature on their efficacy in humans.

The development of effective plant-based products for improving human brain function is constrained by a number of issues, including a need to definitively identify relevant active components and understand synergies within them and an inability to adequately standardize replicable extracts. It is evident that insects such as *Drosophila* and the honeybee are sensitive to modulation by a full range of pharmacological agents. However, insect behavioral studies have only involved secondary metabolites either as a consequence of using them as simple tools for the modulation of specific neurotransmitter targets or alternatively in insect models of drug abuse and addiction (Table 1). It would seem appropriate that insect models could be

utilized as simple, economical, time-efficient, and ethically acceptable tools for investigating the neuronal and behavioral consequences of individual phytochemicals and complex mixtures. It is also evident that there are many viable terpene/ phenolic extracts that may have beneficial effects on CNS function without the toxicity associated with psychoactive alkaloids. These may include complex chemical mixtures that attract symbiotic insects and potentially offer them cognitive benefits. However, many phytochemicals simply do not function effectively as single molecules and there are many examples of synergies within and between the chemical groups. Insect models may provide ideal starting points for disentangling these synergies prior to animal and human studies.

Many secondary metabolites are also expressed as a consequence of environmental stressors, and an increased understanding of the many and varied ecological roles of secondary metabolites should, in the future, make it practical to upregulate and standardize the levels of desired active components by introducing a variety of stressors such as herbivore attack, salinity, UV light, bacteria, or fungi in carefully controlled environments. Finally, the vast majority of the voluminous research relating to the topics briefly reviewed above is conducted in entirely discrete discipline “silos.” In terms of research relevant to brain function, the vast majority is basic laboratory research conducted in vitro/vivo in an entirely atheoretical context, often with parent molecules or chemical concentrations that are highly unlikely to be seen in the human brain. Asking the simple question of why plant chemicals modulate brain function can only serve to focus some of this huge research effort, with the integration of thoughts and concepts from a diverse range of disciplines, including molecular biology/biochemistry, plant science, zoology, entomology, pharmacology, medicine, neuroscience and psychology potentially offering an intellectual synergy that might move this area a step forward.

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Chapter 19

NATURAL HERBS IN STROKE PREVENTION AND TREATMENT

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ABSTRACT

Stroke is the third cause of death and leading cause of chronic disability throughout the world, affecting 15 million people each year. The established risk factors for stroke include hyperlipidemia, dyslipidemia, arterial hypertension, diabetes mellitus, cigarette smoking, micro-vascular rupture, age and observed comorbidity. Although many agents have been used since long for treatment of different cerebrovascular and neurodegenerative diseases but, their effectiveness and safety has attached a great concern from scientists and researchers. Therefore, developing novel classes of anti-inflammatory, anti-oxidative and hypolipidemic neuroprotective agents which possess high efficiency and fewer adverse effects has still been a focus for the preventive treatment of stroke. A variety of herbs and prescriptions have been demonstrated to have neuroprotective effects *in vivo* and *in vitro* that may be relevant to the treatment of stroke. Oxidative stress and inflammation has been increasingly recognized to be the key elements in pathological progression of ischemic stroke. Thus, tackling dyslipidemia, hypertension, reducing oxidative stress and downregulating the inflammatory response are options that merit consideration as potential preventive measures/targets for ischemic stroke. Despite technological developments; herbal drugs still occupy a preferential place in a majority of the population in developing countries and terminal patients in the West. Herbal drugs, in addition to being cost effective and easily accessible, have been used since time immemorial and have passed the test of time without having any side effects. The multi-target effects of herbs (holistic approaches) are the fundamental basis of their

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utilization. This approach is already used in traditional systems of medicine like Ayurveda, which has become more popular in the West in recent years. The treatment of cerebral ischemic disease by natural medicines has a long history, and has accumulated a rich theoretical knowledge and treatment experience. The objective of this chapter is to critically evaluate the experimental research situation of the protective effect of the individual compounds from natural medicine on cerebral ischemia in the recent years, emphasizing the major mechanisms underlying cerebral ischemic pathophysiology. Many representative compounds from natural herbs which are often used to treat stroke are discussed in detail. The results indicate that these components possess a protective effect on cerebral ischemia, and that these components have different mechanisms, including inhibiting excitotoxicity by ginkgolide B, anti-apoptosis of breviscapine, influencing astrocytic activation and proliferation of tanshinone IIA, influencing free radicals by ginsenoside Rd, impairing blood-brain barrier disruption by baicalin, and the anti-inflammatory activity of tetramethylpyrazine. Moreover, some components have multiple neuroprotective mechanisms. Therefore, the combination of individual compounds from natural medicines, considering the mechanisms of cerebral ischemia, may be beneficial to patients with cerebral ischemia in the future. This approach for screening herbs to investigate for prevention and treatment of stroke is a relatively successful method for the identification of herbs and single compounds. Therefore, natural herbs/agents that have a wide spectrum of inhibitory actions on dyslipidemia, hypertension, oxidative stress, inflammation and blood clot dissolution may be useful as preventive medicine and in protecting/rescuing neuronal cells. Here in this chapter we will present a detailed account of the use of natural herbs with multiple effects/targets and different modes of action in reducing the risk factors and prevention of stroke and other cerebrovascular diseases.

We will also evaluate the preventive neuroprotective effect of these plant extracts on reducing the risk factors of stroke and prevent cerebral injury in animal models and humans.

INTRODUCTION

Neurological disorders pose a significant threat in the modern day arena. Cerebral ischemia is the third cause of death and leading cause of chronic disability throughout the world. It involves disruption of the blood flow to the brain with rapid depletion of cellular energy and glucose, resulting in ionic disturbances which in turn initiate a cascade of detrimental events [1]. Stroke has become one of the leading causes of serious, long-term neurologic impairment and functional disability and is the cause of mortality globally. Depending on the severity and type, stroke can leave an individual with a residual damage of physical, psychological, social and cognitive functions [2]. However, there are no known drug therapies to improve recovery after stroke. The established risk factors, including arterial hypertension, diabetes mellitus, cigarette smoking, micro-vascular rupture, hyperlipidemia/dyslipidemia, age and observed comorbidity, such as sickle cell disease, HIV infection and cerebral malaria are increasingly being encountered in the tropics [3].

Cerebral ischemia initiates a cascade of detrimental events including glutamate associated excitotoxicity, membrane lipid degradation, DNA damage, formation of reactive oxygen species and acute inflammation, which lead to the disruption of cellular homeostasis, progressive cell destruction and consequent neurobehavioral impairments. Increased oxidative stress and inflammation has been regarded as an important underlying cause for neuronal damage induced by cerebral ischemia/reperfusion (I/R) injury.

Hence these are increasingly recognized to be the key element in pathological progression of ischemic stroke. In cerebral ischemia and reperfusion (I/R) injury, inflammation is an important pathological process contributing to neuronal cell death and neurovascular injury as well as affecting the progress of neurogenesis and brain repair. Activated inflammatory cells secrete many inflammatory factors including cytokines, chemokines, enzymes, free radicals and other small molecules, subsequently facilitating inflammatory process and accelerating the Blood Brain Barrier (BBB) breakdown, neuronal cell death or affecting brain repair [4].

Therefore, targeting inflammation and related factors could be a crucial therapeutic strategy for preventing and treating brain damage and promoting brain repair. Natural herbs provide a rich source to attenuate lipidemia, neuroinflammation and prevent aggression of cerebral ischemia-reperfusion injury.

Dyslipidemia is characterized by elevated level of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and by lowering level of high-density lipoprotein cholesterol (HDL-C) in serum. Dyslipidemia is one of the major independent risk factors for coronary heart disease (CHD) and stroke [5].

As per American Heart Association (AHA) 2013 Guideline on the Treatment of Blood Cholesterol to Reduce Cardiovascular Risk in Adults” emphasized that the progressively regulating dyslipidemia is the pivotal controlling method for risk factors of ischemic cardiovascular events [6]. Antioxidant and antiinflammatory based drugs and formulations for the prevention and treatment of complex neurological diseases have appeared during last three decades. A number of plant products including polyphenols, terpenes, flavinoids and various plant extracts exert antioxidant, antilipedimic, antiinflammatory actions but till date no effective therapy is available against stroke.

Recent advances in the stroke medicine have highlighted the role of acute transitory inflammation in the cellular pathology following ischemic stroke [7]. It is imperative that acute inflammation might potentiate or perturb the already initiated exitotoxicity. TNF- α has been correlated with cerebral ischemic injury [8]. The advent of inflammation with the proven oxidative conditions can exacerbate the extent of neuronal death. Antioxidant and anti-inflammatory based drugs and formulations from plant sources for the prevention and treatment of complex neurological diseases like stroke, Alzheimer’s and cancer have appeared during last three decades [9]. But till date no drug has been successfully approved for the treatment of stroke therapy. This envisages that there is great scope for finding new therapies for the treatment of cerebral ischemia. Recent studies have shown that a number of plant products including polyphenols, terpenes and various plant extracts exert an antioxidant and anti-inflammatory action [10, 11]. There is also a considerable amount of evidence revealing an association between individuals who have a diet rich in fresh fruits and vegetables and the decreased risk of cardiovascular and other diseases [12, 13].

Despite technological developments, herbal drugs still occupy a preferential place in a majority of the population in the Developing Countries and terminal patients in the West. Herbal drugs, in addition to being cost effective and easily accessible, have been used since time immemorial and have passed the test of time without having any side effects.

The multi-target effects of herbs (holistic approaches) are the fundamental basis of their utilization. This approach is already used in traditional systems of medicine like Ayurveda, which has become more popular in the West in recent years. Natural extracts from genus

Elsholtzia, Scutellaria, Pinax and Artemisia have recently been found to possess strong antilipidemic, antioxidant and anti-inflammatory effects.

Modulation of antilipidemic, oxidative/inflammatory response by these cocktails of natural herbs through different mechanisms in the prevention of stroke may be of vital significance. Here in this chapter we present a detailed account of natural herbal and herbal cocktails in preventing the stroke. Overall, this may help us to understand and find new plant based antilipidemic, antioxidant and anti-inflammatory agents for preventing stroke and other cerebrovascular diseases. A novel herb belonging to critically endangered species Artemisia has been shown to possess very high antioxidant and antiflammatory activity. Very recently Artemesia species from Astereaceae family has been shown to possess anti-oxidant and antiflammatory properties in various in vitro models of cancer and obesity but not against the cerebral ischemia [14, 15]. The plant extract from Artemisia are used locally for the treatment of epilepsy, piles, nervous disorders, cough, cold, fever, and pain [16]. The therapeutic efficiency of some herbs may, in part, be mediated via their influence on the immune response, since some of these plants can affect the immune reactions through their anti-inflammatory actions. The plant had shown anti-inflammatory and also immunosuppressive activity [17].

Other herbs of the genus Pinax (Araliaceae) have been shown to have protective effect on ischemic brain damage in vivo [18] and focal cerebral ischemia [19] Ginseng has neuroprotective effects in transient focal or global cerebral ischemia [20]. Red ginseng powder, which is steamed ginseng under pressure, also prevents delayed neuronal death in gerbils. Ginsenoside Rb1 protects the brain from reversible focal brain ischemia in rats [21] and protects hippocampal CA1 neurons by scavenging free radicals [22].

Herbs of the genus Elsholtzia (Lamiaceae) have a long history of medicinal use in folk. The phytochemical investigations has revealed the presence of flavonoids, phenylpropanoids, terpenoids, and other compounds. Crude extracts from the genus exhibited a wide spectrum of in vitro and in vivo pharmacological activities. As folk medicine, the plants in the genus have been used for the treatment of colds, headaches, pharyngitis, fever, diarrhea, digestion disorder, rheumatic arthritis, nephritises, and nyctalopia in China.

The herb Polygonum cuspidatum (PC) is derived from the dried root and rhizome of Polygonum cuspidatum Sieb. Et Zucc. It dispels wing dampness, removes stagnation, relieves pain, and reduces phlegm. Polydatin and resveratrol, the primary active components of PC, inhibited the cholesterol absorption from intestinal tract [23]. Du et al., found that polydatin administrated orally significantly decreased TC, TG, and LDL-c levels and increased TC/HDL-c ratio in hyperlipidemic hamsters and rabbits [24]. It was also reported that the Polygonum cuspidatum water extract (PCWE) reduced the cholesteryl ester formation in human hepatocytes by inhibiting A-cholesterol acyltransferase activity (ACAT) in HepG2 cell in vitro, and PCWE inhibited ACAT activity by 50% [25].

Recent progress in mass analytic technologies for studying complex systems provides novel platforms for drug discovery from herbal medicine. The comprehensive matrix analytic technologies, such as LC-MS, DNA microarray, proteomics, metabolomics, etc., offers new opportunities to explore the active compounds and their molecular targets, facilitating drug discovery for stroke treatment. Therefore, studies on the therapeutic strategies of herbal medicine not only provide novel clue to find active compounds to prevent hyperlipidemia, neuroinflammation and protect neuronal cells from ischemic brain injury, but introduce a new strategy for drug development targeting multiple neuropathological pathways including

hyperlipidemia, anti-oxidative stress and neuroinflammation in the treatment of ischemic stroke.

Therefore, reducing hyperlipidemia, reducing oxidative stress and downregulating the inflammatory response are options that merit consideration as potential preventive/therapeutic targets for ischemic stroke.

Consequently, agents capable of modulating both elements will constitute promising prevention/therapeutic solutions because clinically effective neuroprotectants have not yet been discovered and no specific preventive therapy for stroke is available to date.

APPROACHES FOR PREVENTION AND TREATMENT OF STROKE

Two major approaches have been developed in ischemic stroke. The first is to establish reperfusion by dissolution of the clot using thrombolytic drugs. At present, rt-PA is the only thrombolytic drug approved for the treatment of acute ischemic stroke; rt-PA administration is restricted to within 3 h of stroke and its use increases the risk of hemorrhagic transformation [26]. The second approach is to develop neuroprotective agents that interfere with the biochemical cascade of events that leads to cell death in the penumbra area that surrounds the core. This protection would attenuate lots of the clinical problems of stroke, including motor disability and spatial hemiplegia. However, although more than 37 potential neuroprotective agents have been studied in more than 114 clinical trials [27], none of them is clinically efficacious and in use in the Western world [28]. In Far Eastern countries such as Korea, China, and Japan, stroke has been treated by traditional Eastern medicine (TEM) for thousands of years. TEM is also known as traditional Chinese medicine (TCM), traditional Korean medicine, Sino-Japanese medicine, oriental medicine, traditional herbal medicine, and traditional Asian medicine. In China, traditional medicine is prevalent; approximately one third of patients are treated with traditional medicine [29], and in Korea, 25% of stroke patients also visit traditional medicine doctors [30]. Extensive experience and abundant clinical data on stroke treatment have been accumulated on TEM. Basic and clinical research in TEM constitutes a potentially rich source of drug discovery and development with the integration of TEM and western pharmacology. In recent years, many attempts have been made to document research data about extracts of composite formulas, single herbs, or single compounds from TEM herbs according to orthodox pharmacological actions. Groups of TEM herbs have been identified as potential sources for compounds with predominant effects on the circulation, thrombogenesis, inflammatory processes, and neuroprotection.

This chapter reviews herbs and prescriptions that have been screened for neuroprotective effects *in vitro* and *in vivo* in ischemic model systems and the neuroprotective compounds isolated from them. Neuroprotective mechanisms of prescriptions, herbs, and single compounds relevant to the treatment of brain ischemia, including antioxidant, antiexcitotoxic, and anti-inflammatory effects are also discussed.

IMPORTANCE OF TRADITIONAL MEDICINE

Traditional medicine (TM) is a comprehensive term used to refer both to systems such as traditional Chinese medicine, Indian Ayurveda and Arabic Unani Medicine, and to various forms of indigenous medicine.

In countries where the dominant health care system is based on allopathic medicine, or where TM has not been incorporated into the national health care system, TM is often termed "complementary," "alternative" or "non-conventional" medicine [31]. The links between TM and biodiversity are exemplified by a long tradition of healing powers associated with the earth's natural systems, whether this entails medicinal plants and animal species, the ambient salubrious air, spring water or the natural scenery. The pharmacopoeia of folk seties as well as professional medical systems like Chinese, Ayurveda, Unani and biomedicine contain thousands of medicines made from leaves, herbs, roots, bark, animal materials, mineral substances and other materials found in nature [32, 33]. The interconnections between TM and the biotic environments may be seen in the health benefits derived from the existence of a full complement of species, intact watersheds, climate regulation and genetic diversity, as well as through our fundamental needs for food, water, clean air, shelter and relative climatic constancy [34]. Discussions of the links between TM and biodiversity therefore are imperative [35], particularly when considering the importance of the former as a source of primary health care to 80 percent of the world's population [36]. The treatment of cerebral ischemic disease by natural medicines has a long history, and has accumulated a rich theoretical knowledge and treatment experience. In recent years, a drive to "modernize" this ancient form of medicine in China has been gaining momentum.

Separation of the chemical constituents of natural medicines and their application to specific health problems (including the treatment of cerebral ischemic disease) is promoted worldwide. With the development of research methods and experimental technology, this process will also broaden mechanism research. Table 4.1 summarizes the studies on various individual compounds from natural medicines that are commonly used in experimental research for the treatment of cerebral ischemia. The following section of the chapter aims to summarize the pathophysiology of cerebral ischemia, and to outline the experimental research situation on the protective effect of cerebral ischemia by individual compounds derived from natural medicines. The various compounds derived from the natural herbs highlighted in Table 4.1 have a great potential for the protection from and treatment of cerebral ischemia. Individual compounds derived from natural medicines, such as ginkgolide B, breviscapine, tanshinone IIA, baicalin, and ginsenoside Rb1 protected from ischemic cerebral injury by the different pathophysiologic mechanisms in experimental studies. This fully illustrates that compounds derived from natural medicines for the protection of cerebral ischemia have broad prospects.

The discovery of the effects of the compounds described here are certainly useful achievements, however, there are also some shortcomings, such as the reproduction of the animal models and the clinical guidance. Furthermore, compound preparation is difficult for the international market due to the fact that the international approval, to date, only applies to treatment with single compounds. Cerebral ischemia is a multifactorial disorder which includes several pathways for the progression of injury to brain cells. Many basic scientists have approached natural medicine as a vast, but largely untapped, source of natural products

in the hope of discovering novel compounds serving as leads for the development of Western-style drugs. Moreover, recognition of each step of the ischemic cascade leads to the possibility of developing a new class of compounds that interferes with a specific mechanism or multiple mechanisms of ischemic injury. Based on the discussion of the sixteen compounds, there is the potential that stroke will become even more treatable, in particular these components have the potential to lead to a reduced permanent disability in patients with stroke.

Table 4.1. Compounds derived from natural medicines used in cerebral ischemia therapy

Source (Latin name)	Monomer Herbal component
Aloe	Aloe polysaccharide
Astragali Radix	Astragaloside IV and Calycosin
Chuanxiong Rhizoma	Tetramethylpyrazine
Crataegi Fructus	Maslinic Acid
Carthami Flos	Hydroxysafflor Yellow A
Chuanxiong Rhizoma	Ligustrazine
Curcumae Longae Rhizoma	Curcumin
Coptidis Rhizoma	Berberine
Erigerontis Herba	Breviscapine and Scutellarin
Ginkgo Folium	Ginkgolide B
Ginseng Radix et Rhizoma	Ginsenoside Rb1, Ginsenoside Rb3, Ginsenoside Rd and Ginsenoside Rg1
Leonuri Herba	Prehispnanolone
Magnoliae Officinalis Cortex	Magnolol
Notoginseng Radix	Maslinic Acid
Polygoni Cuspidati	Resveratrol
Puerariae Lobatae Radix	Puerarin
Picrorhizae Rhizoma	Picroside II
Rehmanniae Radix	Catalpol
Rhizoma et Radix	Resveratrol
Rhodiolae Crenulatae Radix et Rhizoma	Salidroside
Salviae Miltiorhizae	Tanshinone II A
Sinomenii Caulis	Sinomenine
Scutellariae Radix	Baicallin
Ziziphi Spinosae Semen	Jujuboside A

The results reflect significant progress and breakthroughs, which in future, through strong scientific research, have the ability to protect cerebral ischemia by compounds from natural medicines on the basis of the theory of Traditional Chinese Medicine and Western Medicine. Such efforts will also provide a direction for the further application and exploitation of new drug development in the treatment of cerebral ischemia.

Stroke Therapy in Traditional Medicine

Stroke is the first of the four major serious syndromes and the most acute disease in TEM. Stroke in TEM is called ‘wind stroke’ because it happens abruptly like the wind. The concept of stroke in TEM is quite different in many ways from that held by western medicine.

The syndrome is characterized by the sudden appearance of hemiplegia, deviated eyes and mouth, and impeded speech that may or may not start with sudden loss of consciousness. The theoretical systems of TEM are based on the doctrines of yin and yang, the five elements, viscera, and meridian systems. Gong and Sucher nicely reviewed the basic principles and classification of wind stroke in TEM [37].

Generally, the disease state is considered mainly as a destruction of the harmonious components of yin and yang. Wind stroke is considered to be caused either by weak internal strength (so-called ‘qi’) invaded by strong external ‘bad wind’ or by excessive internal ‘fire,’ such as anger, fatigue, heavy drinking, or dietary problems. Both can violate the harmonious negative-positive balance of the self, which eventually leads to stroke.

The treatment of wind stroke in TEM is aimed at creating equilibrium between the relative strength of the patient’s body resistance and the intensity of endogenous and exogenous pathogenic factors. Because wind stroke in TEM is caused by hyperactivity of liver yang, obstruction of the heart orifices by phlegm, excessive heat, or blood stasis.

The treatments in TEM include heat-clearing drugs, anti-rheumatics, drugs for dispersing exterior wind, drugs for promoting blood circulation, drugs for relieving phlegm, drugs for subduing interior wind, drugs for resuscitation, or tonics for deficiency syndromes, in accordance with the cause. Recently, these drugs have been demonstrated to have antioxidant, anti-inflammatory, and anti-glutamate effects. Usually, drugs for clearing away heat, inducing resuscitation, expelling wind, activating blood and removing stasis are used in the early acute stage, and drugs for invigorating and treating deficiencies are used in the later stage.

In the book *Tonggeuibokam*, one of the famous classics in TEM in Korea, 123 different prescriptions for stroke are recorded in the wind stroke section [38]. The herbs used in these prescriptions include, *Saposhnikovia Radix*, *Ligustichi Radix*, *Ginseng Radix*, *Angelicae Sinensis Radix*, *Paeoniae Radix*, *Arisaematis Rhizoma*, *Atylactylodis Rhizoma*, *Notopterygii Rhizoma seu Radix*, *Ephedrae Herba*, and *Scutellariae Radix*, in decreasing frequency. Herbs listed in Table 4.2 are used to treat neurological symptoms of strokes.

NEUROPROTECTION BY INDIVIDUAL HERBS AND THEIR COMPOUNDS

Cerebral ischemic injury is the result of an obstruction of blood flow in a major cerebral vessel, which will lead to a core of severely ischemic brain tissue that may not be salvaged.

However, the ultimate size of the brain infarct also depends on the penumbra, a zone of tissue around the core of the infarct where blood flow is maintained above a neuronal disabling level or the critical 20 to 25% of normal blood flow. Decreased blood flow leads to severe impairment of cellular function by disruption of ATP-dependent processes [39].

Ischemia and subsequent reperfusion provide circumstances that produce oxygen radical production. Several studies have suggested a relationship between cerebral ischemia and oxidative stress in humans [40, 41]. Therefore, antioxidants have been evaluated as neuroprotective agents in stroke [42]. Many herbs, especially those that contain flavonoids, are suggested to have antioxidant effects and possibly might be protective against brain injury caused by ischemia and reperfusion.

Many herbs have also been shown to have anti-inflammatory properties, and thus there is potential for novel anti-inflammatory agents to be identified from plant sources. For example, numerous flavonoid compounds have been associated with anti-inflammatory activity and may have the potential for use in the management of inflammatory disorders [43]. Glutamate toxicity and glucose deprivation is one mechanism of neuronal injury following ischemia. For a thousand years in TEM, some herbs have been used as tranquilizers for their central nervous system (CNS) inhibitory effects. Some of these herbs are known to have neuroprotective effects by the antagonism of excitatory amino acids, particularly glutamate, which is increased in the early post cerebral ischemia period and activates NMDA receptors. Individual herbs are comprised of many compounds, and it is therefore difficult to investigate the exact neuroprotective mechanism of many herbs, even though some show high efficacy in *in vitro* and *in vivo* ischemia models. The effective mechanisms of herbs may include antioxidant, anti-inflammatory, and anti-glutamate actions.

Natural Herbs Extracts and Their Active Compounds

More than 100 herbs have been used for stroke prevention and therapy in TEM. Described below are single herbs and their individual active compounds that have been demonstrated to have neuroprotective effects *in vitro* and *in vivo*.

Ginkgo Biloba

The leaves of *Ginkgo biloba* L. (Ginkgoaceae) have been used in TEM for respiratory and circulatory disorders, and its extract has been therapeutically used for several decades to increase peripheral and cerebral blood flow as well as for the treatment of dementia. *G. biloba* extract EGb761, a standardized extract of *G. biloba* leaves, contains about 24% flavonoid glycosides, primarily quercetin, kaempferol, isorhamnetin, 6% terpene lactones, 2.8 to 3.4% ginkgolides A, B, and C, and 2.6 to 3.2% bilobalide. EGb761 has shown favorable effects on cerebral circulation and neuronal cell metabolism [44, 45] and also exhibited antioxidant activity [46, 47].

EGb761 is neuroprotective against amyloid- and NO-induced toxicity *in vitro*. *G. biloba* extracts attenuate scopolamine-induced amnesia in rats [48], enhance memory retention in young and old rats [49], and improve short-term memory in mice [50]. *G. biloba* attenuates delayed neuronal death in the CA1 of the hippocampus in Mongolian gerbils [51] and is also associated with reduced stroke infarct volume in mice subjected to 45 min of tMCAo [52].

Table 4.2. Showing the some Natural Herbs used in the prevention and treatment of Cerebral ischemia

Name of the Herb	Scientific Name	Alternative names	Family/Group	Type of Neuroprotective effect	Part of herb used	Main components
Ginseng	Pinax Notoginseng	Notoginseng, Sanchi, Three-seven root, Mountain paint	Araliaceae	Anti-inflammatory, antioxidant	Roots	Ginsenosides (Gensenoside Rb1, Gensenoside Rg1), Saponins
Gingko	Gingko Biloba	Maiden hair tree, eun haeng	Ginkgoaceae	Antioxidant	Leaves	EGb761, Bilobalide, Quercitin, Ginkgolides, Keamferol
Scutellaria	Scutellaria baicalensis	Baikal skullcap	Lamiaceae	Antioxidant, anti-inflammatory	Roots	wogonin, baicalin, and baicalein
Salvia	Salvia miltiorrhiza	Red sage, Pinyin	Labiatae	Anticoagulant, Vasodilatory, Anti-inflammatory, Free-radical scavenging, Mitochondrial protection	Roots	Tanshinones
Acanthopanax	Acanthopanax senticosus	Devil's Bush, Touch-me-not, Eleuthero, Siberian Ginseng	Araliaceae	sedative, antioxidant, antihistamine, hypolipidemic, antistress, and immunomodulatory effects	Rhizomes (underground stem) and Roots	eleutheroside, chiisanoside, senticoside, triterpenic saponin, syringin, and flavones
Pueraria	Pueraria lobata	Kudzu	Legumnosae	Free-radical scavenging, anti-lipid peroxidation, and enhancement of SOD activity	Flower and roots	isoflavonoids like Puerarin, daidzin, and daidzein
Ligusticum	Ligusticum chuanxiong	Szechuan lovage Chuang xiang	Apiaceae	antioxidant, antifibrosis, antinociception, anti-inflammatory, antineoplastic	Rhizomes (underground stem) and Roots	Tetramethyl-pyrazine drug
Camellia	Camellia sinensis	Green tea	Theaceae	anti-inflammatory, anti-oxidant	Leaf buds, twigs	Catechins (Epigallocatechin) and flavonols (Theanine).
Rhodiola	Rhodiola rosea	golden root, roseroot, Aaron's rod, arctic root, king's crown	Crassulaceae	Anti-oxidant, antifatigue, antianoxia, and memoryenhancing	Rhizomes (underground stem)	salidroside, rosavin, rosin, rosarin, organic acids, terpenoids
Magnolia	Magnolia officinalis	Houpu, magnolia or magnolia-bark	Magnoliaceae	antihemostatic, antithrombotic, anti-inflammatory, anti-platelet	Root bark, seed, flower	Honokiol and magnolol
Angelica	Angelica sinensis	dong quai, dang gui, tang-kuei	Apiaceae	scavenging effect on peroxide and hydroxyl radicals and inhibitor of lipid peroxidation, Laxative, Sedative	Whole Herb	ferulic acid, ligustilide, angelicide, brefeldin A, butyridenephthalide, butyphthalide
Acorus	Acorus gramineus	Solander	Araceae	induces sedation, potentiates sleeping time, and antagonizes convulsion	Rhizomes (underground stem)	asarone

Name of the Herb	Scientific Name	Alternative names	Family/Group	Type of Neuroprotective effect	Part of herb used	Main components
Paeonia	Paeonia suffruticosa	Mudan, moutan or tree peony	Paeoniaceae	anti-oxidant, analgesic and anti-inflammatory	Root bark	Paeonol, Paeoniflorin
Corydalis	Corydalis yanhusuo	Yan Hu, Cordalis	Papaveraceae	antithrombotic, antihypertensive and anti-inflammatory	Tubers	Protopine, dehydrocorybulbine (DHCB), Tetrahydropalmatine
Huperzia	Huperzia serrata	Firmosses, fir-clubmosses	Huperziaceae	inhibitor of acetylcholinesterase	whole prepared moss	huperzine A
Phellodendron	Phellodendron amurense	Amur cork tree, huáng bò	Rutaceae	anti-inflammatory	Bark	berberine and worenine, flavonoids (diosmin), alkaloids (berberine, yatroriccin, palmatine), saponins and coumarins
Coptis	Coptis japonica	Gold-Thread Mouth-root, Canker-root	Ranunculaceae	antiphlogistic, sedative, antidotal, hemostatic	Rhizome, leaf with stalk, and rootlet	Berberine, magnoflorine, sanguinarine
Uncaria	Uncaria rhynchophylla	Cat's claw, Gambier	Rubiaceae	Antihypertensive, Anti-inflammatory, anticonvulsive, Monoamine Oxigenase-B (MAO-B) inhibitor and anti-spasmodic	Inner bark of the vine or root	Rhynchophylline
Gardenia	Gardenia jasminoides	Jasmine, danh-danh, Gardenia fruit, Cape jasmine fruit	Rubiaceae	anti-oxidant and anti-inflammatory	Fruit	Crocin and Crocetin
Menispermum	Menispermum dauricum	koumori-kazura, bat wine	Menispermaceae	anti-pyretic, analgesic	Roots and Rhizome	Dauricine, Dauriporphine, bisbenzylisoquinoline, aporphine, proporphine, protoberberine, and oxoisoaporphine.
Carthamus	Carthamus tinctorius	safflower plant, Fake Saffron, Alazor, American Saffron	Asteraceae	antioxidant, analgesic, anti-inflammatory and antidiabetic activities	Flower and oil from seeds	carthamin, Carthamidin, isocarthamidin, hydroxysafflor yellow A, safflor yellow A, safflamin C and luteolin
Schisandra	Schisandra chinensis	five flavor berry, Pinyin wi wèi zi, schizandra, gomish	Schisandraceae	Antihypertensive	Fruit	schisandrin, schisandrin derivatives and lignans schisandrol A, B and schisandrin A, B, C,

Bilobalide (Figure 4.1), a sesquiterpene trilactone constituent of *G. biloba*, reduces cerebral edema produced by triethyltin through preventing the uncoupling of oxidative phosphorylation. Pretreatment with bilobalide reduced the cerebral infarct area in MCAo mice [53].

Bilobalide protected the slices against hypoxia-induced phospholipid breakdown [54]. Bilobalide inhibited NMDA-induced phospholipase A2 activation and phospholipid breakdown in rat hippocampal slices [55]. Ginkgolides also had protective effects on focal cerebral ischemia, and its mechanism may be relative to its inhibition of platelet-dependent thrombosis and amelioration of hemorheological apartments [56]. Cumulative evidence indicates that *G. biloba* may have neuroprotective effects in brain ischemia rodent models, and bilobalide may be one of the main compounds responsible for this effect.

There is no convincing evidence from trials of sufficient methodological quality to support the routine use of *G. biloba* extract to promote recovery after stroke [57]. High-quality and large-scale randomized controlled trials are needed to test its efficacy.

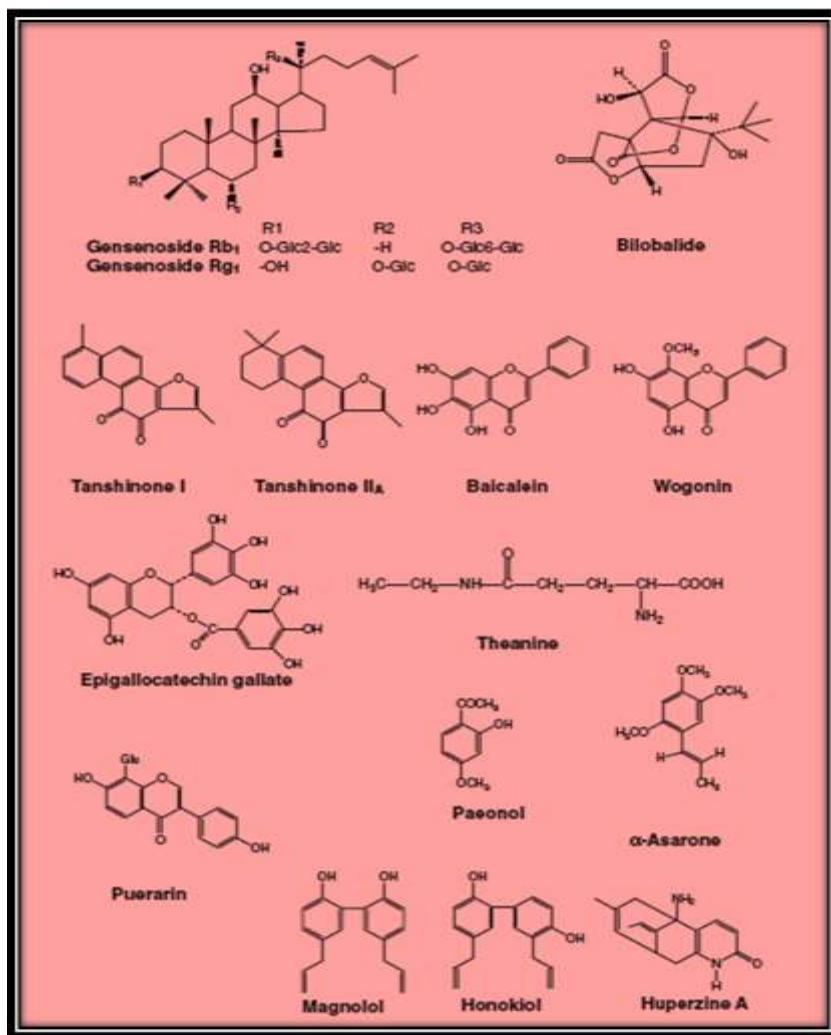


Figure 4.1. Chemical structures of active compounds of neuroprotective herbs.

Scutellaria Baicalensis

The roots of *Scutellaria baicalensis* Georgi (Labiatae) have been used in TEM to treat inflammatory and cardiovascular disease. *S. baicalensis* contains three major polyphenolic components, namely wogonin, baicalin, and baicalein (Figure 4.1). These three polyphenols are as free-radical scavengers of hydroxyl, DPPH, and alkyl radicals [58]. Baicalein is the most effective compound of the three polyphenols tested in preventing glutamate toxicity [59] and is also known as a selective inhibitor of 12-lipoxygenase [60]. Baicalin also acts as a neuroprotectant during cerebral ischemia [61]. *S. baicalensis* and baicalein reduce ischemia-reperfusion brain injury and neutrophil infiltration in MCAo rats [62, 63].

S. baicalensis also protects CA1 neurons against transient 4-VO in rats. It also inhibits microglial TNF- α and NO production *in vitro* [64].

Wogonin, 5,7-dihydroxy-8-methoxyflavone, inhibits ischemic brain injury and improves behavioral dysfunction caused by permanent MCAo [65]. Wogonin has anti-inflammatory activities in various cell types and inhibits NO production by suppressing iNOS induction and NF- κ B activation in microglia [66].

Pueraria Thunbergiana, P. Lobata

The roots of *Pueraria thunbergiana* Benth (Leguminosae) are widely used in TEM for moderating alcohol abuse, for hypotensive, antipyretic, and analgesic effects and for treatment of the common cold. Puerarin (Figure 4.1), daidzin, and daidzein are three of the major isoflavonoid compounds isolated from the extract of *P. lobata*. *P. lobata* flavonoids increase the cerebral blood flow of anesthetized mice [67] and reduce the infarct volume in MCAo by increasing the activities of SOD [68]. Their isoflavonoids have potent inhibitory effects on PGE₂ production [69], and the antioxidant effect, partly dependent on free-radical scavenging, anti-lipid peroxidation, and enhancement of SOD activity [70]. Isoflavones in plants are known to have an estrogenic action. Puerarin has protective effects on cultured mouse cerebral cortical neurons damaged by Glu, NMDA, or KA [71].

Puerarin also shows neuroprotective effects in MCAo rats [72]. Puerarin clearance in normal rats was much faster than that in cerebral ischemia-reperfusion rats induced by MCAo and BCAo [73]. Genistein, a PTK inhibitor, prevented the increase of p-STAT3 and DNA binding activity in ischemic reperfusion injury at 4-VO [74]. It also prevented gerbil transient ischemia via a decrease in tyrosine phosphorylation of NR2B [75].

Magnolia Officinalis

The cortex of *Magnolia officinalis* (Magnoliaceae) has been used for the treatment of acute pain, diarrhea, coughs, and urinary problems in TEM. Honokiol and magnolol (Figure 4.1) are the main constituents of the bark of this herb and have a variety of pharmacological activities. Honokiol has been demonstrated to be an effective antioxidant [76]. It can protect animal tissues against lipid peroxidation [77, 78] serve as an antiplatelet drug [79], and it displays an anti-inflammatory effect in activated macrophages [80, 81]. Honokiol is a potent neuroprotective agent against focal cerebral ischemic injury by its antioxidant and antiplatelet aggregation effects [82, 83].

Magnolol (5,5'-diallyl-2,2'-dihydroxydiphenyl) inhibits intracellular calcium mobilization in platelets [84], relaxes vascular smooth muscle cells [85], and has antihemostatic, antithrombotic [86], anti-inflammatory, and analgesic effects [87].

A number of other effects of magnolol have also been found, including inhibition of prostaglandin D2 formation [87], suppression of nonselective vascular hypo reactivity to mediators [88], reduction of the formation of eicosanoid mediators [89], inhibition of neutrophil adherence [90], prevention of ischemic-reperfusion injury [91], and, most importantly, strong antioxidant activity [92]. Magnolol treatment appears to have a marked effect against heatstroke-induced cerebral ischemic insults [93].

Angelica Gigas, A. Sinensis

Angelicae Radix has been used as a sedative or a tonic and to treat disorders of menstruation in women, anemia, and menopause syndrome. *Angelica gigas* Nakai (Umbellaceae) is used in Korea, and *A. sinensis* (Oliv.) Diels is used in China.

A. gigas includes decursin, decursinol angelate, angelan, and decursinol. *A. gigas* protects mice against β -induced memory impairment [94]. *A. gigas* has antinociceptive effects on pain responses induced by TNF- α , IFN- γ , IL-1 β , glutamate, NMDA, or kainic acid [95]. Decursin ameliorates scopolamine-induced memory impairment in mice [96]. Decursinol and decursin protect primary cultured rat cortical cells against glutamate-induced oxidative stress by both reducing calcium influx and acting on the cellular antioxidative defense system [97]. *A. sinensis* contains ferulic acid, ligustilide, angelicide, brefeldin A, butyridenephthalide, butyphthalide, succinic acid, nicotinic acid, uracil, and adenine.

Extracts of *A. sinensis* showed a scavenging effect on peroxide and hydroxyl radicals and inhibited lipid peroxidation of the liver [98]. *A. sinensis* protects the brain from damage induced by transient forebrain ischemia in mice [99]. *A. sinensis* extract also has attenuating effects on amnesia induced by various drugs related to memory processes [100].

Salvia Miltiorrhiza

The root of *Salvia miltiorrhiza* Bunge (Labiatae) is red in color and was therefore used in TEM to treat related disorders of blood stasis with an action of quickening the blood and dispelling stasis. *S. miltiorrhiza* and its active ingredients, tanshinones and salvianolic acids, have anticoagulant, vasodilatory, anti-inflammatory, free-radical scavenging, mitochondrial protective, and other activities. Experimental studies have shown that *S. miltiorrhiza* dilated coronary arteries and scavenged free radicals in ischemic diseases.

Clinical trials also indicated that *S. miltiorrhiza* was an effective medicine for angina pectoris, myocardial infarction, and stroke [101]. Several studies have investigated possible mechanisms for the protective effect of *S. miltiorrhiza* against cerebral ischemia. *S. miltiorrhiza* attenuated dysfunction of VIP [102] and modified ischemic cell changes by modulating somatostatin [103] in cerebral ischemia. *S. miltiorrhiza* also decreased the size of the infarcted area after CCA ligation in gerbils by inhibiting presynaptic glutamate release and stimulating GABA release [104]. Inhibition of nitric oxide (NO) formation could explain the CNS protective effects observed with *S. miltiorrhiza* [105].

S. miltiorrhiza may offer an additional therapeutic approach to the management of stroke and ischemia. *S. miltiorrhiza* has been shown to offer protection against brain ischemia by reducing lipid peroxidation [106]. Pretreatment with *S. miltiorrhiza* reduced the infarct size in tMCAo-injured SD rats [107]. Tanshinones (Figure 4.1) are the major lipid-soluble pharmacological constituents of *S. miltiorrhiza*.

Brain infarct volume was reduced following treatment with tanshinone IIA and tanshinone IIB in MCAo mice [108, 109]. The therapeutic effect of *S. miltiorrhiza* may be partly due to its free-radical scavenging activities, Tanshinones or other structurally related compounds may have potential for further development as neuroprotective drugs. However, systematic review on randomized control trials comparing *S. miltiorrhiza* with other medicines does not support the notion that *S. miltiorrhiza* may be beneficial to disability improvement after acute ischemic stroke [110].

Acanthopanax Senticosus

The root and stem bark of *Acanthopanax senticosus* Harms (Araliaceae), also called Siberian ginseng, have been used as a tonic and adaptogen to strengthen *qi* in TEM and to treat rheumatic arthritis and stress-induced disease [111]. *A. senticosus* includes eleutheroside, chiisanoside, senticoside, triterpenic saponin, syringin, and flavones in its compounds [112], and it has sedative, antioxidant, antihistamine, hypolipidemic, antistress, and immunomodulatory effects. *A. senticosus* has a protective effect on CCl₄-induced liver toxicity via its antioxidative effect [113, 114]. The saponin isolated from the leaves of *A. senticosus* reduced myocardial infarct size in acutely ischemic dogs [115]. The liriodendrin, syringaresinol, the hydrolysate of liriodendrin, inhibited the LPS-induced production of NO, PGE₂, and TNF- α production by macrophages and decreased expression levels of iNOS and COX-2 enzymes [116]. It has been investigated whether water extracts of *A. senticosus* (100 mg/kg, i.p.) reduced infarct volume in tMCAo of SD rats. In this model, *A. senticosus* inhibited both COX-2 and OX-42 expression in the penumbral region at 24 h after MCAo [117]. These results suggest that *A. senticosus* has a neuroprotective effect via inhibition of inflammation, microglial activation, and antioxidative stress in brain ischemia.

Panax Ginseng

The root of *Panax ginseng* C. A. Meyer (Araliaceae), usually 4 to 6 years after cultivation, has been used as a general tonic or adaptogen and is frequently featured in TEM prescriptions.

Many studies have validated the empirical usage of ginseng over thousands of years for *qi*-invigorating and cardiovascular effects in TEM. The main active components of *P. ginseng* are ginsenosides, which have been shown to have a variety of beneficial effects, including anti-inflammatory, antioxidant, and anticancer effects [118]. *P. notoginseng*, from the same genus as *P. ginseng*, and its saponins exert a protective effect on ischemic brain damage *in vivo* [119, 120] and focal cerebral ischemia [121, 122]. Ginseng has neuroprotective effects in transient focal or global cerebral ischemia [123-125]. Red ginseng powder, which is steamed ginseng under pressure, also prevents delayed neuronal death in gerbils. Ginsenoside Rb1 (Figure 4.1) protects the brain from reversible focal brain ischemia in rats [126] and protects hippocampal CA1 neurons by scavenging free radicals [127]. Ginsenoside Rb1 stimulates the expression of the mitochondrion-associated antiapoptotic factor Bcl-x(L) *in vitro* and *in vivo* [128]. Ginsenoside Rg1 increases ischemia-induced cell proliferation and survival in the dentate gyrus of adult gerbils [129]. With reference to the studies conducted, the neuroprotective effects of ginseng on brain ischemia can be explained by multiple mechanisms, including scavenging free radicals and inhibiting the CNS. The compounds responsible for these neuroprotective effects require further investigation, but ginsenoside Rb1 could be one of the main neuroprotective compounds within the ginseng root.

Gardenia Jasminoides

The fruit of *Gardenia jasminoides* Ellis (Rubiaceae) has been used in TEM for the treatment of inflammation, jaundice, headache, edema, fever, hepatic disorders, and hypertension. Its pharmacological actions, such as protective activity against oxidative damage, cytotoxic effects, anti-inflammatory effect and fibrolytic activities, have already been demonstrated [130, 131].

Crocetin, a major component of gardenia fruits, was found to be a potent inhibitor of tumor promotion via antioxidant activity [132]. Another active component, crocin, exhibited a variety of pharmacological effects in mice, including inhibition of skin tumor growth, improvement of learning behavior previously impaired by ethanol [133], and prevention of long term potentiation caused by ethanol in rats [134]. It could be useful as a treatment for neurodegenerative disorders accompanied by memory impairment. Crocin also combats the serum/glucose deprivation-induced ceramide formation in PC12 cells caused by increasing GSH levels and prevents the activation of the JNK pathway, which is reported to have a role in the signaling cascade downstream of ceramide for neuronal cell death [135].

Paeonia Suffruticosa, P. Lactiflora

The root bark of *Paeonia suffruticosa* Andrews (Ranunculaceae) is a drug used in TEM as both an analgesic and an anti-inflammatory agent [136], and it is prescribed in various TEM preparations for the treatment of blood stagnation.

It has been reported to have strong superoxide and hydroxyl radical scavenging activity [137]. Its antioxidative effects are due to enhancing activities of SOD, CAT, and GPX [138].

Paeonol (Figure 4.1) inhibits cerebral ischemic injury by blocking increases in Ca^{2+} , decreases in SOD activity and the content of MDA, and improved Ca^{2+} -ATPase activity in ischemic brain tissue [139, 140]. A-benzoyloxy-paeoniflorin, an antioxidant monoterpene glycoside, from *P. suffruticosa* has potent radical-scavenging activity on the DPPH radical [141]. The root of *P. lactiflora* Palla has long been used to treat abdominal pain and blood deficiencies in TEM. The oral administration of *P. lactiflora* extract and paeoniflorin, a major constituent of peony root, attenuated spatial cognitive deficits caused by scopolamine in rats [142]. Paeoniflorin reduces the infarct volume as well as ameliorates the deficits in neurological symptoms caused by tMCAo in rats [143]. Paeoniflorin ameliorates memory disruption mediated by adenosine A1 receptor in rodents [144].

Coptis Japonica

The rhizomes of *Coptis japonica* Makino (Ranunculaceae), or *C. chinensis* Franch, have long been prescribed in TEM for the treatment of inflammation-related diseases such as gastrointestinal disorders and infectious or inflammatory diseases. *C. japonica* contains mainly alkaloids, including berberine, magnoflorine, sanguinarine, and phenolic compounds [145]. It has been considered to have antiphlogistic, sedative, antidotal, hemostatic, and antitumor properties. Berberine has been reported to exhibit several types of biological activities, and interest has been focused on its antioxidative potential [146, 147]. *C. japonica* extract and its active alkaloids were effective in an *in vivo* LPS plus ischemia reperfusion model that generated ONOO⁻ [148]. *C. chinensis* administered orally for 1 week improved scopolamine-induced learning and memory deficit in rats [149]. Although the active components of *C. japonica* that exert these bioactivities have not been fully elucidated, it has

generally been considered that its alkaloids, such as berberine, palmatine, and coptisine, contribute to these activities.

Camellia Sinensis (Green Tea)

Green tea contains antioxidant polyphenols such as catechins and flavonols. Most of the experimental and epidemiological studies concerning green tea effects have been targeted at its possible cardiovascular, anti-inflammatory, and anti-carcinogenic effects, which have been linked to the antioxidant/pro-oxidant properties of its polyphenol constituents [150, 151].

Daily ingestion of tea as an antioxidant has also been reported to prevent stroke. Green tea extract orally administered to Wistar rats for 3 weeks before induction of ischemia by occlusion of middle cerebral arteries and reperfusion minimized the eicosanoid accumulation and oxidative damage in addition to the reduction of neuronal cell death [152]. Green tea extract prevented cerebral ischemia damage caused by global ischemia-reperfusion in Mongolian gerbils [153]. (-)-EGCG has potent antioxidant properties in a green tea polyphenol and had a neuroprotective effect against neuronal damage following global ischemia in Mongolian gerbils [154]. Theanine (Figure 4.1), a flavors component of green tea, has a neuroprotective effect against neuronal death in transient brain ischemia. The mechanism of the neuroprotective effect of theanine is related not only to the glutamate receptor but also to other mechanisms such as the glutamate transporter [155].

Huperzia Serrata

Huperzia serrata, a source of huperzine A (Figure 4.1), has been used for centuries in TEM to treat fever, inflammation, blood disorders, and schizophrenia. Huperzine A acts as a potent, highly specific and reversible inhibitor of acetylcholinesterase that crosses the blood–brain barrier. Its potency of acetylcholinesterase inhibition is similar or superior to that of physostigmine, galanthamine, donepezil, and tacrine [156, 157]. Huperzine A protected PC12 cells against OGD-induced toxicity, most likely by alleviating disturbances of oxidative and energy metabolism [158]. Huperzine A treatment is protective against both brain injury and spatial memory impairment in a hypoxic ischemic brain injury of a neonatal rat model [159, 160]. Huperzine A protects against diverse neurodegenerative states observed during ischemia or Alzheimer’s disease by blocking NMDA ion channels [161]. Subchronic oral administration of huperzine A after global ischemia in gerbils significantly reduced memory impairment, reduced neuronal degeneration in the CA1 region, and partially restored hippocampal choline acetyltransferase activity [162].

Acorus Gramineus

The rhizomes of *Acorus gramineus* Solander (Araceae) have been used for the improvement of learning and memory and are often included in the TEM prescriptions for stroke [163, 164]. Water extract or volatile oil from *A. gramineus* induced sedation, decreased spontaneous activity, potentiated pentobarbital-induced sleeping time, and antagonized pentylenetetrazole-induced convulsion in mice [165, 166]. The interactions of *A. gramineus* with the central dopamine (D1 and D2) receptors and the GABA binding site of GABAA receptors were thought to mediate these central inhibitory actions. The methanol extract and the essential oil from *A. gramineus* inhibited excitotoxic neuronal cell death in primary

cultured rat cortical cells [167, 168]. One active principle was identified as asarone (Figure 4.1), a major essential-oil component in the rhizomes of *A. gramineus* [169].

Carthamus Tinctorius

Carthamus tinctorius, the flower of the safflower plant, has been used extensively in TEM for its purported ability to improve cerebral blood flow. *C. tinctorius* protected against excitotoxicity of glutamate, NMDA, kainate, quisqualate and against neuronal degeneration caused by simulated ischemia [170]. *C. tinctorius* exerted significant neuroprotective effects on rats with focal cerebral ischemic injury [171]. *C. tinctorius* reduced cell damage and the formation of peroxidation products after bilateral ligation of the common carotid arteries in rats [172].

Bombycis Corpus

Bombycis Corpus (BC) is a *Bombyx mori* larva (silk moth larva, Bombycidae) killed by infecting with the fungus *B. bassiana*. It has been used in TEM to treat palsy, headache, convulsion, and speech problems induced by stroke and tremor. Several sterols have been isolated from BC. BC has a protective effect against A α -induced cytotoxicity in cultured astrocyte cells through the inhibition of lipid peroxidation and protection of antioxidative enzymes such as catalase, SOD, GSH-Px, and glutathione-S transferase [173].

Sphingolipids from BC also have neurotrophic effects as shown by examining PC12 cell neurite outgrowth [174]. Pretreatment with BC protected primary hippocampal cultures from embryonic day 18 embryos against NMDA-induced toxicity [175]. BC contributes to protect human brain by inhibiting the release of glutamate.

Menispermum Dauricum

Menispermum dauricum DC. (Menispermaceae) roots are used for treating sore throats, colitis, dysentery, and rheumatic arthralgia in TEM. This herb contains some alkaloids belonging to various classes such as bisbenzylisoquinoline, aporphine, proporphine, protoberberine, and oxoisoaporphine. Phenolic alkaloids from *M. dauricum* could attenuate injury caused by left anterior descending coronary artery and BCAo in rabbit by lipid peroxidation and enhance the activity of SOD [176].

Daurisoline, which has been isolated from *M. dauricum*, crosses the blood–brain barrier and will, therefore, facilitate the functional characterization of brain calcium channels in granule cells freshly isolated from rat cerebellum as well as the exploration of P-type calcium channels as possible drug targets [177].

Cnidium Officinale*, *Ligusticum Chuanxiong

The rhizome of *Cnidium officinale* Makino (umbelliferae) is one of the important traditional medicines used for the treatment of female genital inflammatory diseases. *C. officinale* contains a variety of volatile phthalide derivatives that have been shown to have pharmacological activities including sedative, antianemia, antifungal, smooth muscle relaxing, anti-inflammatory, analgesic, and anticomplement activities.

Falcarindiol (1,9-Heptadecadiene-4, 6-diyne-3, 8-diol), which was isolated from *C. officinale*, and the ethyl acetate-soluble fraction of *C. officinale* reduced NO production and suppressed iNOS expression in BV-2 cells and primary microglia cells. The inhibition of

excessive NO production played an important role in neuronal cell death in LPS-treated rat hippocampal slice cultures [178, 179].

The rhizome of *Ligusticum chuanxiong* Hort. has also been used in TCM for the same applications as *C. officinale*. *L. chuanxiong* inhibited platelet activation in bilateral common carotid artery occlusion (BCAo) in rabbits and corrected the TXA2- PGI2 imbalance in plasma after cerebral ischemia [180]. *L. chuanxiong* reduced cell damage-formation of peroxidation products after bilateral ligation of the common carotid arteries in rats [181]. Tetramethylpyrazine, a drug originally isolated from the rhizome of *L. chuanxiong*, has been used routinely in China for the treatment of stroke and angina pectoris. Tetramethylpyrazine has therapeutic potential for the treatment of dementia caused by cholinergic dysfunction and/or decrease of cerebral blood flow. Tetramethylpyrazine pretreatment showed a neuroprotective effect on cerebral ischemia in gerbils [182].

Rhodiola Rosea

The root of *Rhodiola rosea* L. (Crassulaceae) is used as a hemostatic and tonic and for contusions. *Rhodiola* plants demonstrated antifatigue, antianoxia, and memoryenhancing effects. The major compositions of rhizomes of *R. rosea* are phenols such as salidroside and its glycan tyrosol, and cinnamic glycosides such as rosin, rosavin, and rosarin.

Other important constituents are flavonoids, tannins, gallic acid and its esters and essential oils. Administration of *R. rosea* extract for 10 d yielded protection against impairment in memory, as assessed by step-down passive avoidance, induced by electroshock in rats [183]. Several constituents of *R. sacra* and *R. sachalinensis* showed protective effects against beta-amyloid toxicity, oxidative stress, and apoptosis [184]. Phenolic compounds exhibited significant scavenging effects against DPPH free radicals [185]. *R. sachalinensis* treatment reduced infarct volume and attenuated COX-2 induction and microglial activation after tMCAo in rats [186].

Schisandra Chinensis

The fruit of *Schisandra chinensis* Baillon (Schisandraceae) is used in TEM to improve liver and kidney function as an antitussive, tonic, and sedative agent. *S. chinensis* inhibited TBARS formation *in vivo* [187]. The active components of *S. chinensis* are schisandrin derivatives and lignans schisandrol A, B and schisandrin A, B, C, protect primary cultures of rat cortical cells from glutamate-induced toxicity [188]. Pretreatment with schisandrin B also protected against cerebral toxicity induced by tetrabutylhydroperoxide [189].

Corydalis Yanhusuo

The tubers of *Corydalis yanhusuo* W. T. Wang (Papaveraceae) are used in TEM mainly as an analgesic in the treatment of gastric and duodenal ulcer, rheumatism, and dysmenorrhea. *C. yanhusuo* is one of the medicinally important species of *Corydalis*. The tubers are a source of pharmacologically important alkaloids having analgesic [190], antithrombotic [191], antihypertensive [192], and anti-inflammatory effects [193]. Protopine, a component of *C. yanhusuo*, has an inhibitory activity on platelet aggregation [194], and DL-tetrahydropalmatine has a neuroprotective effect in heatstroke-affected rats [195]. It also inhibits calcium anion entry into cells to prevent neuronal death in ischemia-reperfusion rats [196]. It reduced cerebral infarct lesion in focal ischemia-reperfusion injured rats [197].

Phellodendri Cortex

The stem bark of *Phellodendron amurense* Ruprecht (Rutaceae) has long been used in TEM for the treatment of inflammation and fever as a traditional herb medicine having anti-inflammatory, immunostimulatory, and antitumor activities.

It contains some alkaloid components such as berberine and worenine. The antioxidant, anti-inflammatory, and antityrosinase activities of *P. amurense* have been studied [198].

Phellodendri can scavenge superoxide radical (O_2^-) generated through the hypoxanthine-oxidase system and hydroxyl radical OH generated through the Fenton reaction and can inhibit lipid peroxidation induced by the hydroxyl radical generation system [199].

Uncaria Rhynchophylla

The branch of *Uncaria rhynchophylla* (Miq.) Jacks. (Rubiaceae) has been used in TEM for relief of dizziness and treatment of tremors and convulsions [200]. It has antispasmodic effects on smooth muscle and lowers blood pressure [201, 202]. The anticonvulsive effects of this herb have been experimentally demonstrated in KA-treated rats [203].

It inhibited an increase of lipid peroxide levels evoked by ferric-chloride-induced epileptic seizures in rats [204]. Recent studies demonstrated that the extract of *U. rhynchophylla* has a neuroprotective effect on global cerebral ischemia-induced neuronal damage in rats by reduction of COX-2 mRNA and protein level *in vivo* [205].

Rhynchophylline, a major tetraacyclic oxyindole alkaloid isolated from *Uncaria* species, is known to have a protective effect against glutamate-induced neuronal cell death [206]. Both the extract of *U. rhynchophylla* and rhynchophylline ameliorated transient cerebral ischemia-induced spatial memory deficit in mice [207].

Gynostemma Pentaphyllum

Gypenosides, saponins isolated from *Gynostemma pentaphyllum*, are widely used as they are thought to have a wide range of health benefits, including inhibition of inflammation and prevention of cardiovascular disease, due to antioxidant and lipid-lowering properties [208, 209]. Gypenosides decreased injury of DNA and RNA in rat neurons in the 4-VO cerebral ischemia-reperfusion model [210].

Gypenosides suppressed NO synthesis in murine macrophages by inhibiting iNOS enzymatic activity and attenuating NF- κ B-mediated iNOS protein expression, thereby invoking a mechanism by which gypenosides may exert their therapeutic effects [211].

Spiraea Japonica

Spiramine T is an atisine-type diterpene alkaloid isolated from the *Spiraea japonica* var. *acuta* (Rosaceae). It was shown to have neuroprotective effects on cerebral ischemia-reperfusion injury produced by bilateral occlusion of the common carotid arteries in gerbils, and its mechanism might be related to reducing calcium accumulation and lipid peroxidation [212]. Spiramine T reduced the content of lipid peroxide, increased glutathione peroxidase activity, and inhibited the increase of nitric oxidase activity and NO production in the cortex during global forebrain ischemia reperfusion in gerbils [213].

Dioscoreae Rhizoma

The root of *Dioscorea batatas* Decne or *D. japonica* Thunb, called Yam, has been used in TEM for the treatment of diarrhea, cough, dyspnea, leucorrhagia, frequent urination, and diabetes. It is composed mainly of starch with small amounts of mucilage, dioscin, and dopamine.

Dioscoreae Rhizoma was reported to have antisenility [214] and antioxidant activities [215]. My group found that water extracts of *D. japonica* protected against hippocampal cell death in 4-VO of Wistar rats.

Withania Somnifera

Withania somnifera, referred to as *Aswagandha* in the Indian system of medicine, is a central nervous system active herb that has been used for various neurological disorders. Studies with *W. somnifera* have indicated that it exerts an antiaging effect anxiolytic and antidepressant activity [216]. The other pharmacological actions exerted by *W. somnifera* include anti-inflammatory, antistress, hemopoietic immunomodulatory, and antioxidant effects [217, 219]. *W. somnifera* decreased MDA levels and hemispheric lesion area in focal cerebral ischemia induced by MCAo [220].

Curcumin

Curcumin, an active constituent of the rhizome *Curcuma longa*, was demonstrated to have antioxidant potential in many *in vitro* and *in vivo* studies. Curcumin has a neuroprotective effect in tMCAo [221] and bilateral common carotid artery occlusion [222] that is mediated through its antioxidant activity. So a variety of herbs and prescriptions have been demonstrated to have neuroprotective effects *in vivo* and *in vitro* that may be relevant to the treatment of stroke. The majority of *in vivo* studies have been performed in rodent ischemia models, MCAo as focal ischemia, and 4-VO and 2-VO as global ischemia. The mechanisms of neuroprotective herbs in TEM are suggested to be antioxidant, anti-inflammatory, and anti-glutamate effects; however, it is difficult to be precise about mechanisms as the herbs have so many active compounds with disparate mechanisms.

In conclusion, the pharmacological activities of herbs often appear to reflect their traditional uses. The approach for screening herbs to investigate for treatment of stroke is a relatively successful method for the identification of herbs and single compounds.

Future Directions

The development of effective plant-based products for improving human brain function is constrained by a number of issues, including a need to definitively identify relevant active components and understand synergies within them and an inability to adequately standardize replicable extracts. It is evident that insects such as *Drosophila* and the honeybee are sensitive to modulation by a full range of pharmacological agents. However, insect behavioral studies have only involved secondary metabolites either as a consequence of using them as simple tools for the modulation of specific neurotransmitter targets or alternatively in insect models of drug abuse and addiction. It would seem appropriate that insect models could be utilized as simple, economical, time-efficient, and ethically acceptable tools for investigating the

neuronal and behavioral consequences of individual phytochemicals and complex mixtures. It is also evident that there are many viable terpene/phenolic extracts that may have beneficial effects on CNS function without the toxicity associated with psychoactive alkaloids. These may include complex chemical mixtures that attract symbiotic insects and potentially offer them cognitive benefits (106).

However, many phytochemicals simply do not function effectively as single molecules and there are many examples of synergies within and between the chemical groups. Insect models may provide ideal starting points for disentangling these synergies prior to animal and human studies.

Many secondary metabolites are also expressed as a consequence of environmental stressors, and an increased understanding of the many and varied ecological roles of secondary metabolites should, in the future, make it practical to upregulate and standardize the levels of desired active components by introducing a variety of stressors such as herbivore attack, salinity, UV light, bacteria, or fungi in carefully controlled environments.

Finally, the vast majority of the voluminous research relating to the topics briefly reviewed above is conducted in entirely discrete discipline “silos.” In terms of research relevant to brain function, the vast majority is basic laboratory research conducted in vitro/ vivo in an entirely atheoretical context, often with parent molecules or chemical concentrations that are highly unlikely to be seen in the human brain. Asking the simple question of why plant chemicals modulate brain function can only serve to focus some of this huge research effort, with the integration of thoughts and concepts from a diverse range of disciplines, including molecular biology/biochemistry, plant science, zoology, entomology, pharmacology, medicine, neuroscience and psychology potentially offering an intellectual synergy that might move this area a step forward.

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Chapter 20

POLYPHENOLS FROM NATURAL HERBS IN NEUROPROTECTION

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ABSTRACT

Plant polyphenols are dietary components that exert a variety of biochemical and pharmacological effects. Recently, considerable interest has been focused on polyphenols because of their antioxidant, anti-inflammatory, and anti-proliferative activities. Oxidative stress is thought to be a key event in the pathogenesis of cerebral ischemia. Overproduction of reactive oxygen species during ischemia/reperfusion could cause an imbalance between oxidative and anti-oxidative processes. Reactive oxygen species can damage lipids, proteins, and nucleic acids, thereby inducing apoptosis or necrosis. There is increasing evidence supporting the hypothesis that plant polyphenols can provide protection against cerebral ischemia and neurodegenerative diseases. These polyphenols exert their neuroprotective effects through different mechanisms and may function at several cellular levels, including direct interaction and modulation of enzymatic activities and the regulation of signaling pathways with implications for cell survival and death. The mechanisms include the reduction of neuroinflammation via attenuation of the release of cytokines, such as interleukin-1 β and tumor necrosis factor- α and downregulation of the pro-inflammatory transcription factors such as NF- κ B; antioxidant activity, mainly through the inhibition of the NADPH oxidase and subsequent reactive oxygen species generation; an activatory effect on endothelial and inhibitory action on both neuronal and inducible nitric oxide synthase activity and subsequent NO production; and the potential to modulate signaling pathways such as mitogen-activated protein kinase cascade and cAMP response element-binding protein leading to the improvement of memory and cognitive performance. The elucidation of polyphenol activities at the molecular level may lay the foundations for new pharmacological approaches in relation to neurodegeneration. Here in this chapter we will provide a detailed account of different

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polyphenols, their components, molecular mechanisms underlying the potential health promoting effects in relation to cerebral ischemia and other neurodegenerative diseases.

ROLE OF POLYPHENOLS IN NEUROPROTECTION

Phenolics and Polyphenols

Phenolics are ubiquitously found across the plant kingdom, with ~10,000 structures identified to date. Phenolics range from simple low-molecular weight compounds, such as the simple phenylpropanoids, coumarins, and benzoic acid derivatives, to more complex structures such as flavanoids, stilbenes, and tannins. Of these, the flavonoids represent the largest, most diverse group, encompassing some 6000 compounds, all of which share a common underlying structure of two 6-carbon rings, with a 3-carbon bridge, which usually forms a 3rd ring. Flavanoids can then be subdivided according to modifications of this basic skeleton into chalcones, flavones, flavonols, flavanones, isoflavones, flavan-3-ols, and anthocyanins [1].

Research on polyphenolic compounds has started to accelerate with the discovery of the “French paradox” (i.e., the low incidence of coronary heart disease observed in France in association with red wine consumption and intake of a high-fat diet) [2]. This effect seems to be attributed to the polyphenols in red wine. Recently, important epidemiological studies have found that a high consumption of fruits and vegetables can also lower the risk of ischemic stroke [3-5]. The studies regarding health benefits of polyphenols have largely focused on heart disease and cancer. However in recent years polyphenols have got increased attention whether they can offer antioxidant and anti-inflammatory benefits to the brain and have possible preventive effects on stroke and neurodegenerative diseases. For example, initial studies have emerged to show the ability of polyphenolic compounds to improve neurological functions in aging [6-7] and to ameliorate oxidative and inflammatory damages owing to chronic alcohol abuse [8].

A wide range of phenolic compounds in the CNS function, directly interact with neurotransmitter systems. As an example, in animal models, a diverse range of individual and combined flavonoids that occur in traditional medicinal extracts exert sedative/ anxiolytic effects via direct binding to GABAA receptors [9-10], cognitive enhancement via antagonistic GABAA receptor binding and resultant cholinergic upregulation [11], and antidepressant effects via monoamine oxidase inhibition and resultant increases in levels of 5-HT, DA, and noradrenaline in select brain areas [12]. In mammals and other vertebrates, phytoestrogens modulate hormonal systems, and therefore brain function, via a variety of mechanisms [13].

Polyphenols are found in most plant-derived foods and beverages. There are over 8000 polyphenolic structures identified in plants. Polyphenols add to the sensory and nutritional qualities of plant foods. Polyphenols are often involved in the plant's defensive response against different types of stress such as ultraviolet radiation, pathogens, and physical damage. Because plants usually produce these polyphenols as a defensive mechanism, environmental conditions such as soil type, sun exposure, and rainfall along with other factors such as genetics factors, germination, degree of ripeness, processing and storage, and species variety can have effect on the polyphenol concentration. All polyphenols contain an aromatic ring

with one or more hydroxyl group. Most also have at least one sugar residues (glycosides) attached to the hydroxyl groups. They are classified into different groups depending on the number of phenol rings and chemical groups bound to the rings [14-16]. Polyphenols are a group of naturally occurring phytochemicals which are present in high amounts in fruits, vegetables, and natural products and are characterized by the presence of multiple hydroxyl groups on aromatic rings. These compounds are divided into two main categories: the flavonoids and nonflavonoids, based on the number of phenol rings and the way in which these rings interact (Figure 5.1).

Polyphenols contain a wide range of molecule sizes. Polyphenols, such as phenolic acids, are simple compounds, whereas the tannins are highly polymerized molecules. Flavonoids make up most of the polyphenols and they form the most important single group of polyphenols [14]. Table 5. 1 summarizes the main classes of polyphenols, some representative phenolics in the groups, and their dietary sources. Polyphenols are usually recognized for their antioxidant capabilities. Phenolic antioxidants have been shown to inhibit the oxidation of lipids and other molecules and protect against free radicals [14, 17]. Polyphenols can react with radicals to form polyphenol radicals. The polyphenol radical is more stable and less reactive because of the ability of the phenol group to absorb extra electrons. Most polyphenols are conjugated by methylation, sulfation, or glucuronidation during metabolism. The antioxidant capability could be determined by the type of conjugate and its location on the polyphenol structure. This might be why certain polyphenols are better at scavenging superoxides, whereas others can scavenge the highly reactive oxygen-derived radical peroxynitrite. Their antioxidant capacity may also correlate with their ability to chelate metals. Specific polyphenols can chelate iron and possibly prevent the formation of free radicals by iron [18-21].

Polyphenols have been shown to have several other actions in addition to their antioxidant ability. Evidence show that they can inhibit the activities of several enzymes, including lipoxygenase, cyclo-oxygenase, xanthine oxidase, phospholipase A2, ATPases, aldole reductase, phosphodiesterases, topoisomerase I and II, protein kinase C, phosphoinositide 3-kinase, Akt/PKB, (protein kinase B) and mitogen-activated protein (MAPKs) kinases (MAPs) [18, 22-23]. Some polyphenols have weak estrogenic properties and others can inhibit the enzymes involved in estrogen metabolism, aromatase, and 17-hydroxysteroid oxidoreductase [23]. The reduction of several diseases has been linked to polyphenols. Cardioprotection and a reduction in certain types of cancer have correlated with consumption of phenolic antioxidants [14, 23].

There is also evidence for polyphenols to be antiallergic, antiviral, antibiotic, antidiarrheal, antiulcer, and anti-inflammatory agents. Polyphenols have been used to treat hypertension, vascular fragility, allergies, and hypercholesterolemia [14, 16-17]. Polyphenols have also been implicated in the prevention of neurodegenerative diseases.

Polyphenols protect neurons against oxidative stress thought to one of the main causes of neurodegenerative diseases. Even a 10-fold higher concentration of ascorbate did not protect neurons similar to polyphenols [17]. Polyphenols attenuate ischemia-reperfusion injury by interfering with inducible nitric oxide synthase activity, inhibiting lipid peroxidation, decreasing the number of immobilized leukocytes during reperfusion, and reducing complement activation which results in a diminished inflammatory response [18]. Most importantly, in addition to their antioxidant actions, they also influence neuroprotective and neurorestorative signal transduction mechanisms [21]. Epidemiological studies show an

inverse relationship between stroke and polyphenol consumption [15]. The dietary intake of polyphenols varies greatly among different societies. Isoflavone intake as a result of soy consumption ranges from 20 to 240 mg for Asians and from 1 to 9 mg in the United States and Western populations [16, 23].

The evidence presented in this section suggests the potential of polyphenols in both preventive and therapeutic usages for cerebral ischemia/reperfusion injuries. Furthermore, no toxic or other adverse side effects were reported with the dietary use of high concentration of polyphenols, although regulated clinical trials have not been performed. In addition, their bioavailability, absorption, and metabolism also require more studies, especially in humans. It would be particularly important to compare individual polyphenols with extracts of fruits, beverages, and vegetables in preclinical and clinical projects and to further investigate possible mechanisms of their effects. Numerous studies have indicated that compounds in an extract can act synergistically so it would be advantageous to use multiple polyphenols in the treatment of stroke. In particular, when stroke symptoms appear, a substantial damage has already taken place in the brain. Therefore, treatments have to start as early as possible in order to reduce further neurodegeneration and promote regeneration. However, the preventive use of plant products will be likely the most effective strategy for the treatments of stroke and other age-related neurodegenerative disorders.

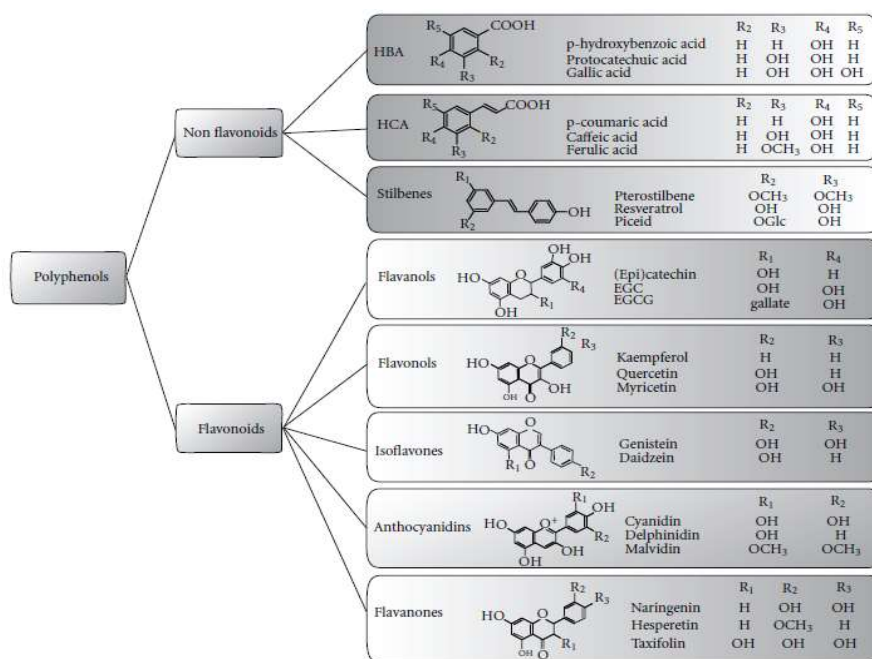


Figure 5.1. Structures of polyphenols. Polyphenols are a group of naturally occurring phytochemicals which are present in high amounts in fruits, vegetables, and natural products and are characterized by the presence of multiple hydroxyl groups on aromatic rings. These compounds are divided into two main categories, the flavonoids and non-flavonoids, based on the number of phenol rings and the way in which these rings interact. For the flavonoid group, the major differences between the individual groups arise from the hydroxylation pattern of the ring-structure, the degree of saturation of the C-ring, and the substitution of the 3-position. HBAs, hydroxybenzoic acids; HCAs, hydroxycinnamic acids.

Table 5.1. Major Subclasses of Polyphenols, Compounds, and Food Sources

Polyphenol Group	Name of Compound	Sources of the Polyphenol compounds
Flavonoids		
Flavonols	Quercetin, myricetin, kaempferol	Onions, apples, kale, red wine, green and black tea, broccoli, berries
Flavanones	Hesperetin, naringenin, eriodictyol	Citrus fruit, tomatoes, mint
Flavanols (Proanthocyanidins and catechins)	Epicatechin, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate	Apricots, green and black tea, red wine, chocolate
Anthocyanins	Cyanidin, pelargonidin, malvidin	Strawberries, other berries, grapes, wine, tea, eggplant, cabbage,
Isoflavonoids (phytoestrogens)	Genistein, diadzein, glycitein	Lentils, chickpeas, alfalfa, clover, flaxseed, soybeans
Flavones	Apigenin, luteolin, diosmetin, tangeretin, nobiletin, sinensetin, wogonin	Parsley, celery, skin of citrus fruit
Phenolic Acids		
Benzoic acid derivative	Gallic acid, vanillic, syringic, hydroxybenzoic	Tea, strawberries, raspberries, blackberries, black radish, onions
Cinnamic acid derivative	p-Coumaric, caffeic, ferulic, sinapic acids, vanillin, syringaldehyde, p-hydroxybenzaldehyde	Blueberries, kiwis, plums, cherries, apples, cereal grains, coffee
Lignan	Secoisolariciresinol	Linseed, lentils, cereals, garlic, asparagus, carrots, pears, prunes
Stilbene	Resveratrol	Grapes, red wine
Saponin	Ginsenoside	Ginseng root
Other polyphenols	Curcumin	Turmeric

Phenolics, and flavanoids in particular, are ubiquitous in plants and therefore represent an important component of a normal human diet. Epidemiological studies have suggested associations between consumption of phenolic-rich foods or beverages and various diseases, such as stroke, cardiovascular disease, and cancer [24] and neurologic disorders such as dementia/AD [25-26]. Naturally, multiple phenolic compounds coexist in foods. Many investigations utilizing animal models have demonstrated, for instance, that berry extracts with high levels of anthocyanins or other polyphenols can reverse brain insult- and age-related cognitive decrements in rodents and that the actives can cross the blood brain barrier [27]. Similarly, in healthy humans, complex mixtures of cocoa-flavanols have been shown to increase peripheral vaso-dilation and cerebral blood flow during task performance, as indexed by functional MRI [28], and improve performance on cognitively demanding tasks [29]. However, the majority of the research in this area is concentrated on the effects of single molecules and the following includes a review of evidence surrounding the 3 most promising single molecule candidates. The chemical structure of curcumin, EGCG and resveratrol are shown in Figure 5.2.

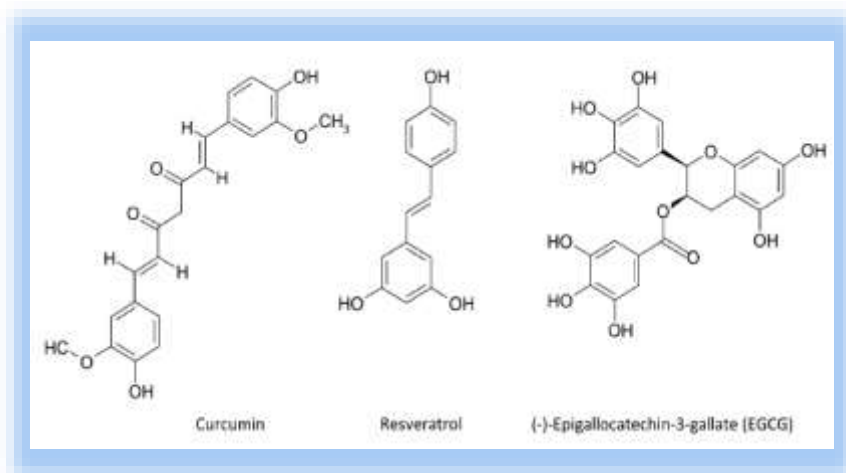


Figure 5.2. Structures of phenolic compounds like Curcumin, Resveratrol and EEG.

Curcumin. Curcumin, a curcuminoid polyphenol responsible for the bright yellow color of the Indian spice turmeric (*Curcuma longa* L.), has been utilized for centuries within the Ayurvedic system of medicine for the treatment of a whole host of ailments, including inflammation [30]. Curcumin exerts varied and wide-ranging effects on molecular targets [31]. These include transcription factors such as NF2, a master regulator of the antioxidant response; the protein kinases, which are involved with the majority of cellular pathways, especially those involved with signal transduction; enzymes such as heme oxygenase 1, a stress response protein whose expression is upregulated after curcumin consumption and associated with neuroprotection [32]; invasion and angiogenesis biomarkers such as matrix metalloproteinase 9, which are associated, among numerous other activities, with tissue repair; and inflammatory mediators such as NF- κ B and cytokines such as TNF α and IL-1 and IL-6 [33]. In animals, some of the physiological effects attributed to curcumin include activity against a range of neurologic diseases in animal models, including AD, multiple sclerosis, Parkinson's disease, age-associated neurodegeneration, schizophrenia and depression [34-39].

EGCG. A number of the catechin polyphenols that are abundant in tea (*Camellia sinensis* L.) are reputed to have pharmacologically active properties. The 4 main tea flavanols are: (-)-epigallocatechin, (-)-epicatechin, (-)-epicatechin-3-gallate, and EGCG, with EGCG generally thought to be the main and active component in green tea. The potentially neuroprotective effects of EGCG include direct effects seen *in vitro* in metal chelation [40], as an anti-inflammatory agent [41], and in the reduction of amyloid- β and amelioration of amyloid- β induced neurotoxicity [42-43], with these neuroprotectant properties being in part mediated via the activation of cell survival genes and modulation of protein-kinase c signaling [40]. EGCG has also been shown to facilitate cholinergic transmission [44], enhance neurite outgrowth [45], and modulate cerebral blood flow parameters in healthy humans. *In vivo* evidence from animal models suggests neuroprotective properties in the face of AD [46-48] and Parkinson's disease [49] and following ischemia/reperfusion injury [50-51]. Long-term administration of green tea catechins (63% EGCG) has also been shown to improve cognitive performance and increase antioxidant capacity in normal rats [52] and rats infused with

amyloid- β [53]. EGCG was also found to significantly increase the lifespan of *Candida elegans*, although, interestingly, this was observed only during situations of increased heat and oxidative stress [54].

This might suggest that the life-extending (and perhaps other) effects of EGCG are due to antioxidant actions and an upregulation of stress resistance-related proteins such as heme oxygenase 1. Indeed, pretreatment of cells with EGCG is associated with an increase in levels of heme oxygenase 1 [55]. Despite the relatively small number of investigations into the neuroprotective properties of EGCG in humans, epidemiological evidence reports that higher consumption of tea/green tea is associated with a reduced risk of neurodegenerative disorders [56] and a lower prevalence of cognitive impairment. Future research should therefore consider both acutely and chronically supplementing green tea catechins to young, healthy participants as well as to those with cognitive senescence.

Resveratrol. The phytoalexin resveratrol (3, 4, 5 trihydroxystilbene) is produced within a range of edible plants in response to tissue damage and environmental stressors such as fungal and viral attack [57-58]. Consumption of resveratrol is associated with numerous protective health benefits in mammals, including increased longevity [59], antiinflammatory [60] and antiviral properties [61], and protection against cancer and tumorigenesis [62], cardiovascular disease [63], and atherosclerosis [64]. With regards to these latter 2 effects, resveratrol has been associated with the French paradox, whereby the consumption of red wine in some cultures has been suggested to contribute to a relatively low incidence of coronary heart disease despite a diet high in saturated fats [65-66]. Potential neuroprotective mechanisms of action include improving blood flow and perfusion [67] and the promotion of antioxidant defenses [68], which *in vivo* are likely to be as a result of resveratrol bolstering the bodies' own endogenous antioxidant defenses via upregulation of a host of antioxidant enzymes [69]. This may be partly a consequence of activation of the Nrf2 transcription factor, which plays a central role in the regulation of cellular redox status [70] and modulation of the protein kinases, which were observed to be involved with neuroprotection against amyloid- β -induced toxicity [71] *in vitro* [72] and *in vivo*, specifically in the hippocampus [73]. *In vivo*, oral administration has also been shown to diminish amyloid- β plaque formation in a region-specific manner in a transgenic mouse model [74]. Resveratrol was shown to provide protection from hypoxic and toxic insults in *in vitro* and *in vivo* experiments of endothelial and primary neuronal cultures (Table 5.2 and Table 5.3).

An expanding body of preclinical evidence suggests that resveratrol has the potential to impact a variety of human diseases. In order to translate encouraging experimental findings into human benefits, more research is needed on the complementary nature of *in vivo* and *in vitro* studies. *In vitro* studies permit rapid screening for interactions, which are likely to be clinically meaningful, and can also be used to evaluate mechanism of action after animal studies; *in vivo* studies confirm or reject the *in vitro* prediction. The vast majority of the published studies on resveratrol performed with *in vitro* or *in vivo* models highlight its potential applications in the prevention and treatment of various disorders through multiple mechanisms of action that may be related to its health benefits [75].

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenolic phytoalexin that occurs naturally in various edible plants [76]. Resveratrol is composed of two aromatic rings connected by a styrene double bond that allows it to exist in *trans*- and *cis*-isomers [77-78]-*trans*-Resveratrol is the preferred steric form and is recognized to have greater biologic activity if it is protected from high pH and UV light. Resveratrol has been identified as a potential factor responsible

for the French paradox [79-80]. In recent years, this molecule has received considerable attention for its anti-inflammatory, anti-apoptotic, anti-oxidative, anti-diabetic, anti-viral and cardio-protective properties [81-84].

Table 5.2. *In Vitro* Examples of Resveratrol in Neuroprotection

In vitro model	Resveratrol treatment	Stress/exposure	Study outcomes	References
Mice primary neuronal cultures	1, 3, or 30 μ M post-treatment for 24 h	OGD for 3 h	neuroprotection	[85]
Rat primary neuronal cultures	0.1, 1.0, or 10.0 μ M pretreatment for 24 h	OGD for 2 h	antiapoptotic	[86]
Rat cortical mixed glial cells	5, 10, 25, 50, or 100 μ M post-treatment for 16 h	OGD for 0.5, 2, or 4 h	neuroprotection and anti-inflammation	[87]
Rat endothelial cultures	100 nM to 10 μ M pretreatment for 3 days	OGD for 3.5 h	antioxidation	[88]
HT22 cells	10 μ M cotreatment for 24 h	4 mM glutamate for 24 h	direct and indirect antioxidation	[89]
PC12 cells	25 μ M pretreatment	50 μ M DTPA-Fe ²⁺ and 1 mM t-BuOOH for 2 h	antioxidation and antimutagenic	[90]
	15 μ M pretreatment for 6 h	H ₂ O ₂ for 12 h	antioxidation, anti-inflammation, anticarcinogenic	[91]
	5, 10, or 25 μ M pre- or post-treatment for 24 h	OGD for 6 h	neuroprotection	[92]
PC12 cells cocultured with BV microglia	100 μ M pretreatment for 3 h	LPS 1 μ g/mL for 24 h	antiapoptotic	[93]
Organotypic hippocampal slice cultures	10, 25, or 50 μ M pretreatment for 24 h	OGD for 1 h	neuroprotection	[94]
Neonatal rat midbrain slice cultures	10–100 μ M	sodium azide, thrombin or N-methyl-N-nitrosourea	neuroprotection	[95]

Table 5.3. *In Vivo* Examples of Resveratrol in Neuroprotection

In vivo model	Resveratrol treatment (route)	Dosing schedule	Study outcomes	References
Neonatal mice (H-I)	0.002, 0.02, or 0.2 mg/kg (ip)	3 different time points: 24 h before H-I; 10 min before H-I; 5 h after H-I	reduced tissue loss	[97]
Mouse MCAO	50 mg/kg (iv)	daily for 7 days	neuroprotection	[98]
	20 mg/kg (po)	acute, 2 h before stroke; chronic, daily for 7 days	neuroprotection from free-radical or excitotoxicity damage	[100]
	50 mg/kg (po)	daily for 7 days before stroke	reduced neuronal injury	[100]
	0.068, 0.68, or 6.8 mg/kg (ip)	single dose after stroke	neuroprotection with extended therapeutic window	[85]
	1, 2.5, or 5 mg/kg (iv)	3 or 6 h after stroke	neuroprotection and anti-inflammation	[101]
Rat MCAO	20 mg/kg (po)	for 3 days after stroke	neuroprotection	[102]
	30 mg/kg (ip)	first dose 3 h post-stroke and subsequent daily dose for 4 days	neuroprotection and antiapoptotic	[103]
	30 mg/kg (ip)	pretreated for 7 days	neuroprotection	[104]
	0.001 or 0.003 mg/kg (iv)	single dose 15 min before ischemia	vasodilation, antioxidation	[105]
	0.001 or 0.003 mg/kg (iv)	single dose at reperfusion	antioxidation	[106]
	0.1 mg/kg (iv)	twice, 15 min preocclusion and at the time of reperfusion (2 h postocclusion)	reduced infarct volume and edema, improved neurological deficits, anatomical and functional preservation	[107]
	15 or 30 mg/kg (ip)	pretreated for 7 days and 30 min before MCAO	reduced neurological score, infarct volume, and brain water content	[108]
	20 mg/kg (ip)	pretreated for 21 days	decreased infarct volume and antioxidation	[109]
	oxy-resveratrol, 10 or 20 mg/kg (ip)	twice, immediately after MCAO and at reperfusion	reduced infarct volume, improved neurological deficits	[110]
Rat SAH	10 mg/kg (iv)	daily for 3 days after hemorrhage	neuroprotection, antioxidation and vasodilation	[111]
Rat global ischemia	10, 50, or 100 mg/kg (ip)	pretreated for 48 h	neuroprotection	[112]
Rat recurrent ischemic stroke	25 mg/kg (po)	three doses of resveratrol given daily after stroke	neuro- and cardio-protection	[113]
GerB BCCAO	30 mg/kg (ip)	twice, during and shortly after CCA occlusion	decreased delayed neuronal cell death and glial cell activation, can cross blood-brain barrier	[114]

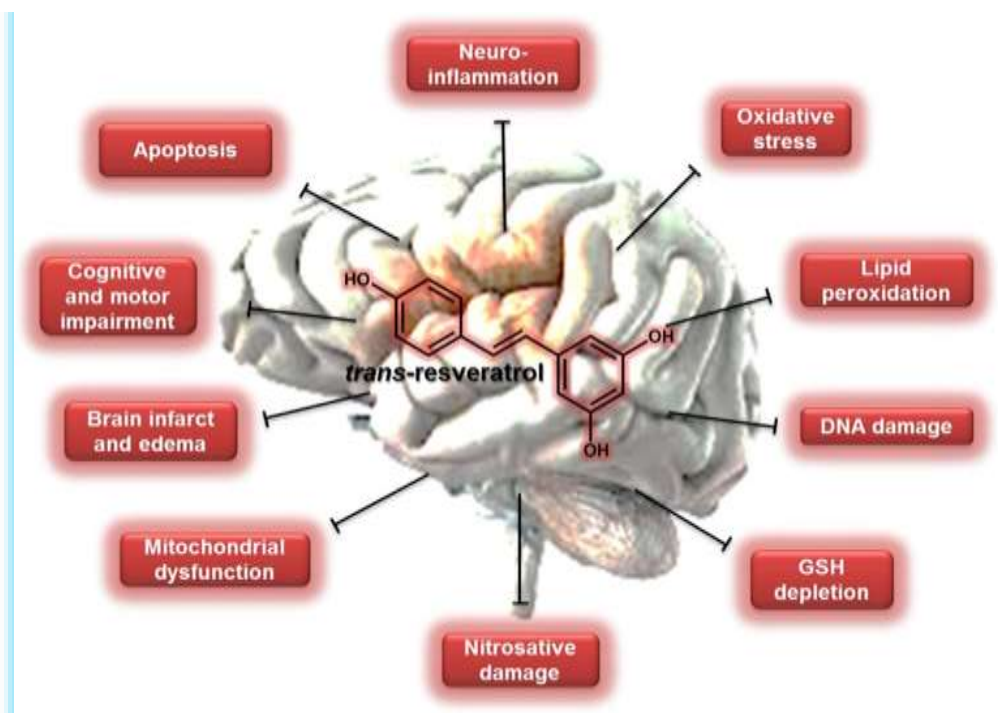


Figure 5.3. Potential targets associated with anti-stroke activity of resveratrol.

Resveratrol exhibits therapeutic response against stroke by preventing brain infarct, edema, mitochondrial dysfunction and cognitive and motor impairment. Furthermore, it diminishes nitrosative, oxidative, and DNA damage, which leads to preclusion of apoptosis and neuroinflammation.

Here, in this section we discuss the findings on the neuroprotective potential of resveratrol from *in vitro* and *in vivo* stroke experimental models and multiple mechanisms of action that may be related to its health benefits through either direct or indirect antiapoptotic, anti-inflammatory, and antioxidative routes (Figure 5.3). This summary also helps to clarify the relationships among *in vitro* potency with respect to mechanism of action, drug concentration, and *in vivo* efficacy in clinical and preclinical findings.

Resveratrol, a natural stilbene present at relatively high concentrations in grape skin and seeds and red wine, is known for its purported antioxidant activity in the vascular and nervous systems. In contrast to its direct antioxidant role within the central nervous system, recent research supports a protective mechanism through increasing endogenous cellular antioxidant defenses, which triggers a cascade of parallel neuro-protective pathways.

A growing body of *in vitro* and *in vivo* evidence indicates that resveratrol acts through multiple pathways and reduces ischemic damage in vital organs, such as the heart and the brain, in various rodent models. Most of the protective biological actions of resveratrol have been associated with its anti-oxidative, anti-inflammatory, and anti-apoptotic properties and other indirect pathways see figure 5.3.

PROTECTIVE ROLE OF POLYPHENOLS IN NEURONAL ISCHEMIC INJURY

A significant interest on the protective effects of polyphenols has principally been because of their antioxidant properties. Phenolic antioxidants have been shown to inhibit the oxidation of lipids and other molecules and protect against free radicals [114]. Oxidative stress is a key event in the pathogenesis of cerebral ischemia. Overproduction of ROS during ischemia and/or ischemia/reperfusion can damage lipids, proteins, and nucleic acids, thereby inducing apoptosis or necrosis. Increasing evidence supports the hypothesis that plant polyphenols provide protection against neurodegenerative changes associated with cerebral ischemia [115].

Whether regional differences exist in the brain in the protective effects of polyphenols in ischemic injury is not clear. Most studies have reported the protective effects of polyphenols in the hippocampal and cerebral cortex regions in ischemia. Inanami et al. [116] observed a dose-dependent protection against hippocampal neuronal death in ischemia in gerbils after ad libitum oral administration of catechin in the drinking water for 2 weeks. Epigallocatechin-3-gallate (EGCG) also protected the hippocampal region in gerbils after transient global ischemia [117] and neuronal damage in a rat model of transient focal cerebral ischemia [118]. EGCG (50 mg/kg; intraperitoneal) was effective even when it was administered 3 hr after the ischemic insult in gerbils [119]. Hong et al. used green tea extract in the drinking water ad libitum for 3 weeks before ischemia in gerbils. This treatment reduced the infarct volume, the number of apoptotic cells, and lipid peroxidation, and inhibited the ischemia-induced hyperactivity [120]. In another focal ischemia model using middle cerebral artery occlusion (MCAO) in rats, the protective effects of resveratrol were shown with pretreatment for 21 days (20 mg/kg intraperitoneally per day). The treatment reduced the infarct volume, prevented motor impairment, and inhibited lipid peroxidation [121]. A single dose of resveratrol (20 mg/kg) given orally 1 hr before permanent middle cerebral artery ligation in mice did not protect against ischemic damage. However, when given daily for 3 days before ischemia, resveratrol significantly reduced the infarct size. In another study, effects of resveratrol on transient global cerebral ischemic injury were examined in gerbils [122]. Resveratrol (30 mg/kg given intraperitoneally per day) was injected either during or shortly after common carotid artery ligation and 24 hr later. Resveratrol significantly decreased neuronal death in the hippocampus and also inhibited glial cell activation. Nanocapsule encapsulated quercetin treatment resulted in significant protection to endogenous antioxidant enzymes against ischemia induced oxidative damage in neuronal cells of young and old rats [123]. Not many studies have reported the effects of polyphenols in the striatum except that by Shukla et al. [124] who saw a significant inhibition in lipid peroxidation and an increase in superoxide dismutase (SOD) activity in corpus striatum in rats pre-treated with curcumin prior to MCAO.

Some studies have examined the protective effects of polyphenols in the striatum but not in ischemic injury. For instance, GTE and EGCG were effective in preventing the depletion in striatal dopamine and tyrosine hydroxylase protein levels in a mouse model of Parkinson's disease [125]. It appears from these studies that protective effects of polyphenols would potentially be observed in the striatal region if assessed in ischemic injury. As mentioned above, cerebral cortex is another region where ischemic injury has been observed. Shukla et

al. [124] reported an antioxidant effect of curcumin in the cortex of rats subjected to MCAO. Red wine polyphenol compounds also protected against oxidative stress in rats following MCAO/reperfusion [126]. Similarly, resveratrol significantly attenuated neuronal death in and decreased the generation of ROS, lipid peroxidation and nitric oxide (NO) content in the cortex of rats subjected to transient global ischemia [127]. 2,3,5,4'-tetrahydroxystilbene- 2-O-beta-D-glucoside (TSG), an active component of the rhizome extract from *Polygonum multiflorum*, significantly reduced infarct volume in the cortex following MCAO [128]. Taken together, these studies indicate that polyphenols either exert or have the potential to exert neuroprotective effects in various regions in the brain that are vulnerable to ischemic injury. While the precise dose required to achieve a neuroprotective effect in cerebral ischemia is not clear and may vary with individual polyphenols,

Sutherland et al. [118] have reviewed the effects of green tea catechins including the safety and efficacy of such catechins. One mechanism underlying the neuroprotective effect of polyphenols is possibly through its effects on reducing the levels of apoptotic markers. Pomegranate polyphenols and resveratrol protect neonatal mouse brain from ischemic injury by reducing caspase-3 and calpain activation [129]. In neonatal rats, amentoflavone blocked the activation of caspase-3 and the proteolytic cleavage of its substrates following hypoxic-ischemic injury [130]. Pomegranate juice also diminished caspase-3 activation in the hippocampus and cortex of the neonatal brain against a hypoxic-ischemic insult through supplementation of the maternal diet with pomegranate juice [131]. Mangiferin and morin, two antioxidant polyphenols, are neuroprotective in both *in vitro* and *in vivo* models of ischemia possibly by reducing Ca^{2+} influx and decreasing caspase-3 [132]. A subsequent *in vitro* study by Campos-Esparza et al. [133] demonstrated that mangiferin and morin reduced the formation of ROS and restored the mitochondrial membrane potential following excitotoxic stress, which is a major component of ischemic injury. Further, these polyphenols also reduced the glutamate-induced activation of calpains, normalized the level of cytosolic Bax and inhibited the release of AIF from mitochondria. These actions of mangiferin and morin could well be part of their profile in an *in vivo* model of ischemic injury. EGCG, a green tea polyphenol, reduced up-regulation of MMP-9 activity and neuronal damage following transient focal cerebral ischemia in C57BL/6 mice [134]. MMP-9 downregulation by resveratrol was also observed in an *in vitro* model of neuronal ischemic injury [135]. 5,7,3',4',5'-pentahydroxy dihydroflavanol-3-O-(2"-Ogalloyl)-beta-d-glucopyranoside (AP1), a polyphenolic compound isolated from *Anogeissus pendula* Edgew (an arid forest tree), was effective in reducing apoptotic cells in rat brain following transient focal cerebral ischemia [136]. The effect of TSG in protecting rat brain from MCAO is by increasing the anti-apoptotic Bcl-2 proteins.

Curcumin, a potent polyphenol antioxidant enriched in turmeric, reduced cytochrome c release and subsequent caspase-3 activation following global cerebral ischemia in Mongolian gerbils [137]. While the aforementioned studies have demonstrated a decrease in caspase-3 levels in the presence of polyphenols, it is unclear whether polyphenols act directly on caspase-3 or whether they act on upstream caspases that are precursors to caspase-3. Alternatively, such polyphenols could also be activating inhibitor of apoptosis (IAP) which would then inhibit caspase-3 activation. In addition, effects of polyphenols may also involve protecting mitochondrial dysfunction in ischemic injury as seen *in vitro* [138]. Preventing the decline in mitochondrial membrane potential following ischemic injury may subsequently confer protection against apoptotic cell death. In addition, resveratrol can induce

neuroprotection by increasing mitochondrial ATP synthesis efficiency in rat brain following ischemia [139]. While these studies highlight the potential neuroprotective mechanisms by which polyphenols attenuate cell death in ischemia, their antioxidant and anti-inflammatory effects may also contribute to their ability to reduce cell swelling and/or brain edema which can be deleterious to neuronal and glial functioning.

ROLE OF POLYPHENOLS IN ATTENUATING OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION

Oxidative stress is a key component of ischemic injury including cell swelling and brain edema and polyphenols, due to their antioxidant properties, would be postulated to attenuate such injury. Reports on the beneficial effects of polyphenols on brain edema in ischemia are scarce. Resveratrol has been reported to reduce brain edema in rats following MCAO [140]. Lee et al. has reported a protective effect of green tea polyphenol EGCG against neuronal damage and brain edema after unilateral cerebral ischemia in gerbils. AP1, a polyphenolic compound, also reduced brain edema in rats after transient focal ischemia [141]. Recently the protective effects of polyphenols from green tea as well as cinnamon on glial swelling in cultures following ischemia-like injury has been reported [142]. Myricetin and quercetin also attenuated cell swelling following oxygen-glucose deprivation in C6 cultures [143]. While in cell culture studies polyphenols reduced cell swelling, it is possible that the reduction in cell swelling was not due to the antioxidant effects of polyphenols.

An increase in intracellular calcium is a key feature of ischemic injury [144]. Further, an increase in $[Ca^{2+}]_i$ can induce cell swelling as demonstrated in lactic acidosis-induced glial swelling [145] and in hypo-osmotic swelling in cultured astrocytes [146]. It has been demonstrated that quercetin and myricetin both attenuate OGD-induced increase in $[Ca^{2+}]_i$. Also, such blockade of the rise in $[Ca^{2+}]_i$ by blockers of the L-type calcium channel as well as modulation of $[Ca^{2+}]_i$ through BAPTA, a calcium chelator, reduces cell swelling in C6 glial cultures [147]. Other studies have also shown a decrease in $[Ca^{2+}]_i$ following administration of polyphenols. Quercetin attenuated the H_2O_2 -induced calcium dysregulation in PC12 cells [148]. Quercetin, catechin, and resveratrol also inhibited cardiac voltage gated sodium channel in rat cultured myocytes, but had no effect on the reverse mode NCX, the Na^+ / Ca^{2+} exchanger [149]. Apple condensed tannins inhibit the increase in intracellular free Ca^{2+} concentration in RBL-2H3 cells induced by antigen stimulation [150]. EGCG reduces the glutamate-induced $[Ca^{2+}]_i$ increase by attenuating ionotropic Ca^{2+} influx in PC12 cells [151]. Nevertheless, these studies indicate that polyphenols have the potential to modulate calcium channels that are involved in cell volume regulation, but their role in attenuating glial swelling/cytotoxic edema in ischemia needs to be further elucidated.

Mitochondrial dysfunction is an important characteristic of ischemia. The mitochondrial permeability transition (mPT) has been implicated as one mechanism, or at least part of the mechanistic pathway, for cell swelling in cultured astrocytes following ammonia toxicity or TBI as well as in brain sections in ischemia [152]. Despite these studies, the role of the mPT in cell swelling is not clear. Recently it has been demonstrated that the attenuation of cell swelling and the prevention of the decline in mitochondrial inner membrane potential ($\Delta\Psi_m$) by immunosuppressants, cyclosporin A (CsA), but not FK506, are consistent with the role of

the mPT mediating such events. Similar to CsA, CPE, and green tea polyphenols, also significantly prevented OGD-induced cell swelling and the decline in $\Delta\Psi_m$ in C6 glioma indicating that one mechanism by which CPE and GTE exert their protective effects is possibly by blocking the mPT. Interestingly, quercetin significantly attenuated cell swelling in C6 glial cells following OGD but did not block the dissipation of the $\Delta\Psi_m$ [147] indicating that other factors, besides the mPT, mediate the development of cell swelling in ischemic injury. It is also possible that preventing the induction of the mPT may be sufficient in some cases but may not be always necessary.

An increase in inflammatory markers has been associated with brain edema [153] and could potentially cause damage to the BBB [154]. A disruption of the BBB is observed in vasogenic brain edema. A key characteristic of polyphenols is their anti-inflammatory property [155] and anti-inflammatory effects of polyphenols have been reported in cerebral ischemia [156]. Inflammatory molecules can damage mitochondrial function. For instance, exposure of rat astrocyte cultures to interferon $-\gamma$, in the presence or absence of LPS, can increase NO production which can subsequently damage the mitochondrial respiratory chain complex function. Studies that investigated the role of polyphenols on BBB function in ischemia are scarce except that reported by Zhang et al. [157] which examined the effects of green tea polyphenols on BBB permeability following MCAO in rats. They report a decrease in BBB permeability in the ischemic region in the presence of green tea and a concomitant decrease in levels of caveolin-1, a protein involved in BBB functioning and permeability. Wang et al. and Lee et al. report a reduction in water content in the brains of animals following ischemia with resveratrol and EGCG respectively, but it is not clear if the edema that was measured was of the vasogenic or cytotoxic type. Likewise AP1, a polyphenolic compound, also reduced brain edema in rats following MCAO but the type of edema assessed is not clear. A reduction in BBB damage and water content in the brain following cerebral ischemia in rats was reported with curcumin [158]. Curcumin also decreased brain edema in rats following MCAO [159] but as with some other studies the type of edema examined is not clear. In a rat thromboembolic stroke model, curcumin reduced brain edema [160] most likely of vasogenic type.

In addition, curcumin was reported to significantly lower oxidized proteins and interleukin-1 β , a pro-inflammatory cytokine, elevated in the brains of AD transgenic mice [161]. IL-1 receptor 1 (IL-1R1)-null mice when subjected to hypoxia-ischemia showed reduced cytotoxic and vasogenic edema when compared to wild-type mice [162]. Taken together it is conceivable that curcumin could attenuate vasogenic edema following ischemia. Further, anti-inflammatory properties of polyphenols have been reported in other stresses and this knowledge can be applied to vasogenic edema in ischemia. Polyphenols found in cinnamon also have anti-inflammatory effects *in vitro* [163]. A reduction in TNF- α , an inflammatory cytokine, has been reported for green tea polyphenols [164] as well as dried plum polyphenols [165] and TNF- α is one agent that increases endothelial permeability in vasogenic edema. Also, increases in intercellular adhesion molecule (ICAM-1) and myeloperoxidases in rodent lung injury are attenuated by green tea polyphenols [166]. In addition, anti-cyclooxygenase 2 effects of resveratrol [167], as well as anti-MMP9 effects of resveratrol [168] and other polyphenols have been demonstrated. The importance of inflammation in vasogenic edema, taken together with the anti-inflammatory effects of polyphenols, indicates that the polyphenols may play a protective role in reducing vasogenic brain edema in ischemia.

OXIDATIVE STRESS AND MITOCHONDRIAL DYSFUNCTION ARE KEY FEATURES OF CEREBRAL ISCHEMIA

Oxidative stress and mitochondrial dysfunction are key features of cerebral ischemia that affect neuronal viability after ischemia. In addition, these factors also affect brain edema which is a major consequence of ischemia and can be fatal if not resolved. Edema can further aggravate neuronal injury by affecting cerebral perfusion. Currently, there are few remedial agents to effectively reduce neuronal death or brain edema not only in ischemia but also in other neural injuries including traumatic brain injury. The potential for the use of polyphenols in the preventing cell loss or damage and edema in cerebral ischemic injury is tremendous. However, the cellular and molecular actions of polyphenols involved in neuroprotection have to be elucidated further. Given the large proportion of the population affected by stroke and traumatic brain injury, and with few strategies to effectively attenuate brain edema and associated neural damage, it is important to determine the potential beneficial effects of dietary polyphenols in the prevention and alleviation of such damaging effects.

ROLE OF POLYPHENOLS IN PREVENTING NEUROINFLAMMATION

Although neuroinflammation plays a critical role in brain host defence, it also contributes to the underlying neuronal loss in neurodegenerative disorders and to damages associated with cerebral ischemia [169]. Neuroinflammation is “driven” by activated resident glial cells (astrocytes and microglia) which result in invasion of circulating immune cells and the production of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6), nitric oxide (NO), prostaglandin E2, chemokines, and reactive oxygen species (ROS). Amongst the numerous factors released by activated glial cells, excessive NO• production has been reported to induce neuronal cell death by damaging the mitochondrial electron transport chain function in neurons [170] therefore resulting in neuronal ATP synthesis disruption and in increased generation of ROS [171]. Furthermore, NADPH oxidase activation, an important event in activated microglia-induced neurotoxicity, has also been suggested to mediate both superoxide (O₂⁻) production and to release proinflammatory molecules such as TNF- α [314]. NO• produced in microglia or astrocytes may react with O₂⁻, produced by NADPH oxidase to generate the neurotoxic peroxynitrite radical (ONOO-) [172]. ONOO- has been observed to inhibit mitochondrial respiration, induce caspase-dependent neuronal apoptosis, and to induce glutamate release resulting in excitotoxicity and neuronal death [173]. Additionally, glial cytokine production may also play a deleterious role in neurodegenerative diseases by binding to specific cell surface receptors expressed in neurons and activating apoptotic pathways.

There has been much interest in the development of new drugs capable of preventing neuroinflammatory-mediated brain injury. Emerging evidence suggests that dietary polyphenols may exert neuroprotective effects by suppressing the activation of microglia, which mediates inflammatory processes in the CNS. Although rather complex, the main anti-inflammatory properties of polyphenols include: (1) an inhibitory role on the release of cytokines, such as IL-1 β and TNF- α , from activated glia; (2) an inhibitory action against iNOS induction and subsequent nitric oxide production in response to glial activation; (3) an

ability to inhibit the activation of NADPH oxidase and subsequent ROS generation in activated glia; (4) a capacity to downregulate the activity of proinflammatory transcription factors such as NF- κ B through their influences of a number of glial and neuronal signaling pathways, such as MAPK cascade (discussed in details below) [174-175]. For example, the commonly consumed flavonol quercetin has been reported to inhibit neuroinflammation by attenuating nitric oxide production and iNOS gene expression in microglia [176] and by preventing inflammatory cytokine production, thus preventing neuronal injury [177-178]. However, one of the major physiological metabolites of quercetin, quercetin-3_-sulfate, failed to demonstrate any anti-inflammatory action. Nevertheless, these studies have employed quercetin concentrations (10–50 μ M) much higher than of those found in plasma after ingestion.

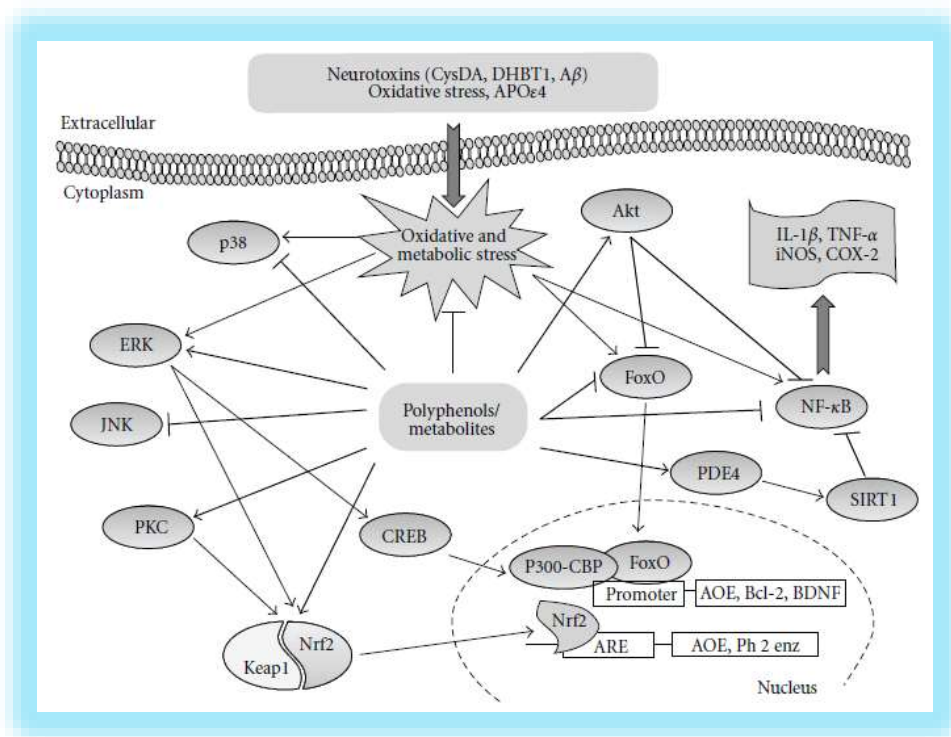


Figure 5.4. Mechanisms underlying the biological effects of polyphenols. Polyphenols and their *in vivo* metabolites activate cellular stressresponse pathways resulting in the upregulation of neuroprotective genes. For example, both PKC and ERK can activate the nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 then translocates to the nucleus and binds to the antioxidant response element (ARE) in genes that encode cytoprotective proteins such as antioxidant enzymes (AOE) and phase 2 (Ph2) enzymes. The transcription factor cAMP-responseelement-binding protein (CREB) is also activated by ERK, which induces the expression of brain-derived neurotrophic factor (BDNF), a mediator of neurohormesis. In addition, polyphenols can also regulate the transcription factor NF- κ B, which can mediate adaptive cellular stress responses by reducing the expression of inflammatory cytokines. Activated SIRT1 may also inhibit NF- κ B and so can reduce the cellular stress response. Another important pathway activated bymetabolic and oxidative stress involves transcription factors of the forkhead (FoxO) family, which modulate genes that encode antioxidant enzymes and other stress-response proteins.

In contrast to this, epicatechin and catechin (10–300 nM) were observed to inhibit TNF- α release but not iNOS expression or nitric oxide production in primary glial cells [179] suggesting that flavanols at physiologically relevant concentrations may hold the potential to exert anti-inflammatory effects in the central nervous system. Polyphenols present in blueberry have also been reported to inhibit NO $\bullet$, IL-1 β and TNF- α production in activated microglia cells [180], and the flavanone naringenin was observed to be highly effective in reducing LPS/IFN- γ -induced glial cell activation [179]. Dietary polyphenols are also potent inhibitors of NADPH oxidase activity *in vitro*. A study comparing 45 polyphenolic compounds indicated that whilst both the flavanols (+)-catechin and (–)-epicatechin failed to inhibit NADPH oxidase, their relevant methylated metabolites exhibited strong NADPH oxidase inhibition through an apocynin-like mechanism [181]. Interestingly, other apocynin-like phenolic compounds, such as, ferulic acid, homovanillin alcohol, caffeic acid, tyrosol, and vanillic acid were also observed to inhibit NADPH oxidase activity, therefore indicating that smaller polyphenols, more structurally related to some colonic metabolites, may also serve as novel therapeutic agents in neuroinflammation (Figure 5.4).

There is also data which shows encouraging positive effects of polyphenols in animal and *in vitro* models relevant to multiple sclerosis (MS), a chronic debilitating disease which is characterised by demyelination, progressive irreversible axonal damage and inflammation [182]. For example, EGCG delivered orally reduces symptom severity in the autoimmune encephalomyelitis model of relapsing remitting MS by reducing inflammation and increasing neuroprotection [183]. Quercetin has also been reported to be effective in the Experimental Autoimmune Encephalomyelitis (EAE) mouse model, and reduces T-cell proliferation *in vitro* at concentrations exceeding 10 μ M [184]. Micromolar concentrations of luteolin, apigenin, fisetin, and quercetin (but not morin or hesperetin) were reported to suppress the production of the cytokine interferon-gamma (IFN γ) from lymph-node-derived T cells but, paradoxically, worsen clinical severity in the EAE model. More recently, resveratrol protection against EAE was associated with rises in IL-17/IL-10 and with repressed macrophage IL-6 and IL-12/23 p40 expression [185]. Thus, the studies to date show promising proof of concept of beneficial effects of polyphenols in suppressing immune and inflammatory responses in models of MS.

NEUROPROTECTION BY POLYPHENOLS IN HYPOXIC-ISCHEMIC INJURY IN NEONATES

Dietary supplementation with foods rich in polyphenols—pomegranates, blueberries, green tea, and apple juice—has been shown to provide neuroprotection in animal models of focal brain ischemia, of periventricular white matter injury, and of Alzheimer's disease [186–189]. Polyphenols have been found to possess antioxidant properties as well as to have effects on gene expression [190]. Specifically, one polyphenol, resveratrol, has been shown to increase activity of members of the sirtuin gene class, blunting p53 action and blocking apoptosis [191–192]. Recent studies indicate that among foods that contain polyphenols, juice extracted from the pomegranate has the highest concentration of measurable polyphenols [193–194]. The pharmacologic actions of pomegranate juice include antiatherosclerotic, antibacterial, and antiproliferative properties [195]. We recently found that when the

polyphenol rich pomegranate juice is consumed by the dam polyphenols from the juice are present in the pup and protected the pup against H-I brain injury [131]. Other studies have shown that the polyphenols caffeic acid, phenylethyl ester and amentoflavone are also protective against neonatal H-I brain injury [130]. To test the hypothesis that it is the polyphenols of pomegranate juice that are responsible for neuroprotection, we tested the effect of pomegranate polyphenol extract (PPE) in the neonatal H-I mouse model. Supplementation of PPE to the drinking water of pregnant and nursing dams resulted in significantly decreased H-I induced caspase-3 activation. This suggests that it is the polyphenols of the pomegranate juice that are responsible for the neuroprotection.

To further investigate the role of polyphenols in neonatal H-I we focused on the specific polyphenol resveratrol. This naturally occurring compound has been found to be neuroprotective in adult ischemia in rats when administered before the injury, but to our knowledge resveratrol has never been tested in neonatal H-I [196-198]. By examining a variety of different concentrations at several different time points we found that IP injection of resveratrol leads to decreased caspase-3 activation in the P7 mouse in a concentration and time dependent manner. At doses of 200 $\mu\text{g/kg}$ or greater, resveratrol leads to decreased caspase-3 activation but only when resveratrol is injected prior to the injury. In addition to decreasing the caspase-3 activation resveratrol also decreases the calpain activation following neonatal H-I, suggesting that it works as a generally neuroprotective agent and not just on the apoptotic pathway.

In addition to finding that resveratrol is protective in the neonatal mouse we also demonstrated that resveratrol protects the neonatal rat against H-I induced caspase-3 activation. Although the injury paradigm is similar in rats and mice there are several neuroprotective agents that have been found to work only in one species. Since resveratrol has been found to protect against stroke in neonatal rats and mice as well as in adults, it could potentially be considered for further investigations in humans. Interestingly, we did not find resveratrol to be protective in the rat when given after the injury. Since the apoptotic cell death in the rat starts much later in the rat than in the mouse, and several drugs have been shown to be protective in the rat when given after the insult, we thought that resveratrol might follow the same pattern. The fact that resveratrol does not protect when given after the injury suggests that it is acting through proximal mechanisms in the cell death pathway initiated by H-I. One pathway that may be involved in the effects of polyphenols is via activation of the sirtuins such as SIRT1.

Polyphenols such as resveratrol may have beneficial effects on health via their antioxidant properties, suppression of inflammatory pathways, or other pathways such as activation of the sirtuin pathway [199]. Included in the sirtuin family is SIRT1, a human protein deacetylase that promotes cell survival by mechanisms such as negatively regulating the p53 tumor suppressor [200], deacetylating the transcription factor FOXO3 [201], repressing PPAR γ signaling [202] and modulation of NF- κ B dependent transcription [203]. Modulation of these pathways may provide a means to protect the developing brain against neonatal H-I induced brain damage. Recent studies show that polyphenols, including resveratrol, increase cell survival via activation of SIRT1. Parker et al. found that increased *sir2* gene dosage or treatment with resveratrol in *C. elegans* blocked neuronal dysfunction and cell death induced by polyglutamine expansion. Suggesting that resveratrol may act through a similar pathway in mammals, resveratrol protected mammalian neuronal cell lines from mutant huntingtin-induced cell death and this effect was inhibited by sirtuin

inhibitors [204]. There is also evidence that resveratrol can block axonal degeneration via SIRT1 in the mammalian peripheral nervous system [205]. While increasing evidence suggests that resveratrol and other polyphenols are neuroprotective, whether their protective actions in the CNS *in vivo* are via SIRT1 has not been directly assessed. Determining the mechanism of protection of resveratrol, pomegranate polyphenols, and other polyphenols may lead to novel insights into both pathogenesis and treatment of neonatal H-I brain injury.

SUMMARY

The neuroprotective actions of dietary polyphenols involve a number of effects within the brain, including a potential to protect neurons against injury induced by neurotoxins, an ability to suppress neuroinflammation, and the potential to promote memory, learning, and cognitive function. While many of the mechanisms underpinning their beneficial effects remain to be elucidated, it has become clear that they in part involve decreases in oxidative/inflammatory stress signaling increases in protective signaling, and may also involve hormetic effects to protect neurons against oxidative and inflammatory stressors. Most of the dietary polyphenols that have been shown to be protective against age-related disease are all chemically reactive and nearly all are electrophilic.

Such chemical features renders these molecules capable of influencing the redox potential of their target cells and to modulate series of transcriptions factors that result in the activation of phase I and phase II metabolism genes. Nonetheless, much of the data obtained on their bioactivity derived from short-term basis *in vitro* or *in vivo* studies where the dose used was not of nutritional relevance. Although at the moment, the balance of evidence that does suggest that polyphenol effects contribute to the benefits of a high intake of fruits and vegetables, the extent of their contribution *in vivo*, and at physiological relevant concentrations remains uncertain. More work needs to be done to prove whether this class of compounds is most likely to result in health benefits and to determine their beneficial effects in slowly developing neurodegenerative disorders. In view of their multiple biological activities, the consumption of polyphenol-rich foods throughout life holds a potential to limit neurodegeneration and to prevent or reverse age-dependent deteriorations in cognitive performance. However, the therapeutic and pharmacological potential of these natural compounds still remains to be translated in humans in clinical conditions. Moreover, efficacy in RCT is also needed to support the relatively consistent epidemiological and mechanistic evidence. Despite this lack of efficacy data and the uncertainty of their effects *in vivo*, investigations into the absorption and metabolism of various polyphenols in humans indicate that there are common pathways for the metabolism of the majority of polyphenols, notably via their bacterial metabolism in the large intestine. Consequently, research on developing dietary polyphenols for applications in neurodegenerative disorders should prioritise investigations of smaller polar polyphenols for brain bioavailability and bioactivity. The challenge ahead therefore is to proceed cautiously until rigorous randomized controlled clinical trials have been undertaken to determine empirically whether polyphenols and/or their metabolites have efficacy in individuals affected by dementia and other neurodegenerative conditions.

In general, the literature on the efficacy of the herbal extracts and phytochemicals reviewed here in terms of improving aspects of human brain function is somewhat equivocal. Research on two alkaloids, caffeine and nicotine, is confounded by withdrawal effects and most of the remaining treatments have failed to progress beyond relatively small scale human studies. Indeed, in the case of the single molecule polyphenols (curcumin, resveratrol, EGCG), their huge and exponentially expanding literatures are singularly lacking in reports of relevant human intervention trials. Of the 3 treatments that have progressed to larger scale controlled trials and eventual meta-analyses, both GB and valerian are be devilled by methodological inconsistencies and inadequacies that make conclusions difficult to draw, with only St. John's Wort consistently demonstrating efficacy.

The phenolic compounds, particularly those like flavonoids that are ubiquitously consumed in plant-based foods, may then owe the balance of their CNS effects to the latter (but with notable exceptions in terms of hormonal effects and GABAergic effects). As well as the natural compatibility of molecules created by conserved stress signaling pathways common to both plants and humans, it is interesting to note that the induced antibacterial/fungal and viral effects of curcumin, EGCG, and resveratrol within the plant may be mirrored by a similar protection conferred after exposure to similar pathogens in human cells and animal models. Although an exact concurrence between the mechanisms of action across the taxa has not yet been established, Friedman has demonstrated that, *in vitro*, the antibacterial, antitoxin, antiviral, and antifungal properties of tea flavonoids were similar against all of the food-borne pathogens reviewed.

These mechanisms ostensibly involved either binding to the invader and inactivating it or perturbing the membrane structure of the pathogen and causing leakage, with both resulting in preventing or limiting the deleterious effects of the bacteria, toxin, or virus. With phenolic compounds in particular it is also interesting to note that humans are likely to have lost the ability to synthesize vitamins, which include several terpenoids and methylated phenols, because the ubiquity of these micronutrients in our diet made it more advantageous in evolutionary terms to sequester them from food rather than synthesize them *de novo*. The same argument has been made for all dietary antioxidants, including many non-vitamin phytochemicals, and this proposition could be extended to include the non-antioxidant properties of groups of phytochemicals that occurred as part of our natural ancestral diet. This would largely accommodate the phenolic compounds, and flavonoids in particular, that are ubiquitous in plant foods. It may be relevant that most phenolic compounds have low parent molecule bioavailability but still exhibit *in vivo* bioactive effects. The rapid process of metabolism that takes place in the body could be viewed as the body processing the molecules into, for instance, glucuronidated and sulfated metabolites to more effectively transport and utilize them, in much the same way that vitamins are processed into their active metabolites and derivatives following consumption.

The gradation in toxicity and ecological/CNS functions is also seen in the comparative levels of research attention paid to the chemical groups. The alkaloid group has benefitted from intense research for over 200 yrs and has provided a multitude of medicinal compounds with CNS activity. Interest in terpenes, on the other hand, has really only escalated in the last 25 yrs, during which time many advances have been made in terms of characterizing the constituents and activities of complex plant extracts that often have low toxicity, high bioavailability, and a multitude of potentially relevant physiological effects. Similarly, research into the health effects of phenolic compounds has only reached any considerable

level within only the last 15 yrs. In the case of alkaloids, they have proven particularly amenable to research and drug discovery because of their comparatively straightforward, single molecule modes of action. Evidence suggests that extracts with largely terpene or phenolic actives owe their effects to multifarious synergies between their component chemicals and this factor, along with an inability to reliably standardize extract constituents, has to date constrained their development and the clarity of the literature on their efficacy in humans.

The development of effective plant-based products for improving human brain function is constrained by a number of issues, including a need to definitively identify relevant active components and understand synergies within them and an inability to adequately standardize replicable extracts. It is evident that insects such as *Drosophila* and the honeybee are sensitive to modulation by a full range of pharmacological agents. However, insect behavioral studies have only involved secondary metabolites either as a consequence of using them as simple tools for the modulation of specific neurotransmitter targets or alternatively in insect models of drug abuse and addiction. It would seem appropriate that insect models could be utilized as simple, economical, time-efficient, and ethically acceptable tools for investigating the neuronal and behavioral consequences of individual phytochemicals and complex mixtures. It is also evident that there are many viable terpene/ phenolic extracts that may have beneficial effects on CNS function without the toxicity associated with psychoactive alkaloids. These may include complex chemical mixtures that attract symbiotic insects and potentially offer them cognitive benefits. However, many phytochemicals simply do not function effectively as single molecules and there are many examples of synergies within and between the chemical groups. Insect models may provide ideal starting points for disentangling these synergies prior to animal and human studies.

Many secondary metabolites are also expressed as a consequence of environmental stressors, and an increased understanding of the many and varied ecological roles of secondary metabolites should, in the future, make it practical to upregulate and standardize the levels of desired active components by introducing a variety of stressors such as herbivore attack, salinity, UV light, bacteria, or fungi in carefully controlled environments. Finally, the vast majority of the voluminous research relating to the topics briefly reviewed above is conducted in entirely discrete discipline “silos.” In terms of research relevant to brain function, the vast majority is basic laboratory research conducted *in vitro/vivo* in an entirely atheoretical context, often with parent molecules or chemical concentrations that are highly unlikely to be seen in the human brain. Asking the simple question of why plant chemicals modulate brain function can only serve to focus some of this huge research effort, with the integration of thoughts and concepts from a diverse range of disciplines, including molecular biology/biochemistry, plant science, zoology, entomology, pharmacology, medicine, neuroscience and psychology potentially offering an intellectual synergy that might move this area a step forward.

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Chapter 21

RECESSION, SKILLS AND NATIONAL DEVELOPMENT: THE SOUTH AFRICAN CASE

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ABSTRACT

When an entity's profitability is threatened, skills development initiatives are subjected to stress. This is not unusual in unusual times. In trying economic times, the firm-level skills development initiatives take second place as a result of the austerity measures either in place already or in preparation for its anticipated effects. But what happens to national development imperatives in these times? This chapter explores the impact of the 2008 global financial crisis on national and skills development in South Africa. It reports the results of a firm-level response to employability and skills development and juxtaposes this with the national level response to the crisis in the country. Using data gathered at the height of the crisis, it is contended that the responses by national government and at firm-level, while reacting to the immediate needs of the economy, did little to impact on the substantive equity imperatives to which the country strives. In light of the peculiar skills development challenges, much more could have been achieved in holding private companies accountable for equity in light of their call for assistance. The 'golden moment' offered by the crisis represented an opportunity lost in the pursuit of workplace transformation as the need for redress and equity becomes increasingly urgent in South Africa.

Keywords: National development, Skills transfer, Equity and transformation, Workplace transition, Development and recession

JEL Classification: C12, C33, G01, G11, G21.

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INTRODUCTION

When an entity's profitability is threatened, skills development initiatives are subjected to stress. This is not unusual in unusual times. In trying economic times, the firm-level skills development initiatives take second place as a result of the austerity measures in place or anticipated in the foreseeable future (see for instance Kluve, 2006; TUC, 2009). But what happens to national development imperatives in these times? This chapter explores the impact of the 2008 global financial crisis on skills development for a selection of South African companies in the manufacturing sector. It reports the results of a firm-level response to employability and skills development undertaken during the economic crisis in light of the peculiar skills development challenges facing the country. It is argued that while the peculiar context allows for reliance on the state in responding appropriately to the crisis, the impact of the recession on equity and transformation is likely to be muted. What was an opportunity afforded by the crisis to achieve more equitable outcomes, has been lost.

Countries have responded in various ways to the crisis. Some states have chosen to make education integral to their 'recovery plans' (Bray and Varghese, 2009, p. 2), while others, such as the BRIC (Brazil, Russia, India and China) countries, have made education a critical component of semi-protected budgets (Douglass 2010, p. 24). More importantly, national governments have gone out of their way to encourage firms to retain their staff and to stimulate skills development in an attempt to mitigate the worst social impacts of the crisis (OECD 2009, p. 30). According to the OECD, the investment in education and training not only makes displaced workers less vulnerable, it also contributes to national economic restructuring as displaced workers find new job opportunities and support this new national restructuring (OECD 2009, p. 10). Such measures are therefore aimed at sustainably protecting the most vulnerable from the full impact of economic meltdowns. There is, nevertheless, need for ever vigilant social support, protection and extension, where the education sector is threatened by declining national budgets (Barakat et al. 2010). As such the upside to the downturn is that it does provide scope for countries to reposition themselves in the skills arena and offers an opportunity to engage more meaningfully with the equity and access imperatives in their economies. Therefore, one of the key challenges in circumstances like these is to capitalize on the moment with a sustainable and integrated response. Clearly, a coordinated response requires the imperatives of displacement to be accompanied by a surge in national education funding. However, in a situation where declining national budgets offer little additional funds, more creative responses are called for to engage the crisis.

While a coordinated and integrated response might well have required both supply-side imperatives (state education and training entities) and demand-side constituents (public entities and private sector companies) to be locked into synergy, there has been a perceptible lack of any engagement between these elements. However, in the South African case, the lack of synergy was more pronounced as national development challenges become more serious.

This chapter argues that the call for state support by private sector companies presented an opportunity for the establishment of more equitable workplaces. Especially since those that were most likely to be most affected by the recession were beneficiaries of the broader transformation agenda. By exploring the key features of the policy response to the recession and company-level survey data on skills development practices and recruitment, it is suggested that an opportunity might well have been lost to effectively engage the transformational imperative in South Africa. While some jobs might have been saved, the

impact on workplace equity could have been much more deliberately advanced. An opportunity was, therefore, lost.

The chapter begins with an overview of national development challenges. This is followed by the impact of the recession on key features of the South African economy. The response of the South African government is then explored. The chapter closes with some critical insights regarding implications of the response to the recession on the need to advance the equity imperative necessary for national development.

NATIONAL DEVELOPMENT CHALLENGES IN SOUTH AFRICA

National development challenges have been profoundly shaped by South Africa's fractured past and are still associated with considerable race, class, and related issues linked to its colonial legacy. The concern with the inequity that still prevails is everywhere apparent.

The excessive social challenges which the country faces include inter alia high unemployment, inequity, crime, corruption, ineffective social structure and poor service delivery. They suggest that unless some serious review of the social democratic transition is undertaken, the success of the national transition is unlikely. Various national policy proposals have been identified to resolve these challenges in the post-apartheid era. These include the Reconstruction and Development Program (RDP) introduced at the time of the transition in 1994; the Growth, Employment and Redistribution Strategy (GEAR) introduced in 1996 which was replaced by the Accelerated and Shared Growth Initiative (AsgiSA) (see McGrath and Akoojee, 2007). The latest initiative is the National Growth Path (NGP), initiated in 2010, which accompanies the Human Resource Development Strategy (HRD-SA) in providing the basis for a human-centered growth path. The latter has been particularly useful in attempting to focus attention on the need for national development to have a peculiarly inclusive nature. The African National Congress (ANC), the ruling party, has been particularly sensitive to the prevailing concern of inequity and has, for instance, been vociferous about the need to ensure that the latest initiative succeeds: "We will also make sure that these projects lead to more inclusive growth by meeting the needs of new industries and historically excluded communities" (ANC, 2011).

The equity imperative in society as a whole, and in education, has been a key consideration of the policy arena. Given a historical legacy of poor education access at every level, there has been a pressing need to ensure that generally poor skills level, low standing in international social development measures and the unusually high poverty levels need special consideration in the national developmental context (Bhorat and Kanbur, 2006).

Inequity is especially endemic. South Africa is ranked amongst the most unequal countries in the world. The Gini coefficient for the country is currently at 0.578 (World Economic Forum, 2011) with significant racial disparities, which makes South Africa one of the most unequal countries in the world. The 2009 Development Indicators Report points out that the Gini coefficient reached 0.666 in 2008 and 0.679 in 2009 (Pressley, 2009).

Survey results also reflect the wide disparity in society. The Income and Expenditure Survey conducted in 2005/6 reported that while 10 per cent of the population earn more than 50 per cent of household income, the poorest 40 per cent of the population account for less than 7 per cent of this, with the poorest 20 per cent accounting for less than 1.5 per cent

(StatsSA, 2008). Although it has been pointed out that poverty had begun to decline between 2001 and 2005 (Woolard and Woolard, 2008), the reality that the problem is a result of structural factors that need deliberate and targeted response cannot be ignored.

Rampant inequity is also associated with an unusually high unemployment rate. Current unemployment on the outer end estimates is pegged at between 27 and 30 per cent nationally. Strict unemployment peaked in 2001 at 31 per cent. There was reported growth in ‘broad unemployment’, as it did finally make inroads into unemployment, falling to 23 per cent (NPC 2011, p. 10). The current unemployment in the broad definition is estimated at 25 per cent for the third quarter of 2011. The problem of youth unemployment is particularly disturbing. One report estimated that there are more than 2.8 million people, aged between 18 and 24, who are not in education, employment or training (referred to as NEETs) (Cloete, 2009).

Education and skills development capacity is also consistent with employment. It has been reported that the probability of gaining employment is significantly increased once a tertiary qualification is attained (Bhorat et al. 2010). Despite the fact that there are considerable demographic, institutional and program choice considerations that hinder the prospects of some of those that qualify, it is evident that social progress is unlikely to be achieved for many without significant advances in post-school education and training access (see for instance Akoojee and Nkomo, 2007). The need for the country to advance the social development agenda by effective and meaningful investment in education at all levels is abundantly clear.

The need to ensure that the country succeeds is evidenced by the establishment since 2009 of a specialist National Planning Commission tasked with the responsibility of ensuring that a systemic response to the challenges is both developed and monitored.



Figure 1. Nature of the development challenge in South Africa. Source: NPC 2011, p.7.

The diagnostic report of this commission, released early in 2011, identified the centrality of educational outcomes and its counterpart, unemployment, as a key feature of the challenge facing the country as the Figure 1 reproduced from this document shows.

The challenge of poor and ineffective education outcomes is central to other key developmental challenges including inter alia employment, corruption, structural economic inconsistencies (resource intensiveness and poor service delivery by public entities).

The nature of the national development challenges in South Africa is therefore considerable. Poverty, inequality and unemployment loom large in the national arena, with its associated skills deficits and shortages in key areas of the country.

RECESSION AND ITS IMPACTS: THE SOUTH AFRICAN STORY

Impact of the Crisis

The onset of the economic recession as a result of the sub-prime crisis in the United States resulted in considerable international consternation. The subsequent government interventions and bailouts of macro-capitalist institutions that followed had important implications for the skills and national development. Importantly, it halted economic growth which averaged between 5 and 7 per cent globally before the crisis. The global economic hemorrhaging as a result of the decline in trade led to significant job and income losses and resulted in considerable setback of social programs of most governments (Chitiga et al. 2010; Verick and Islam, 2010). According to estimates by the International Labor Organization (ILO), it was to lead to more than a million people losing their jobs, and in World Bank estimates, an additional 46 million people globally were pushed below the poverty line (quoted in Bray and Varghese, 2009). While developed countries have been quite significantly affected, developing countries, especially those dependent on commodity prices, have been under tremendous strain. The resultant declining trade flows led to falling remittances from developed nations and impacted negatively on their already steadily dwindling aid budgets.

South Africa, although initially cushioned from the recession, was not completely unaffected. It could be argued that in the period from November 2009 to February 2010, the most detrimental impacts were to some extent neutralized by the government spending on infrastructure in preparation for the impending FIFA Football World Cup. But the initial ripples of the world economic turmoil were already being felt. The country was, nevertheless, initially insulated from its most visible deleterious results. In addition to the infrastructural spending of the international sporting event in 2010, the relative cushioning of the economy as a result of the banking regulations introduced before appeared to have had some positive impacts. The National Credit Act of 2008 for instance softened rampant lending. On the flip side, this resulted in a degree of lethargy about a meaningful response to the crisis and its likely impact on South Africa. As Hein Marais points out, “... until well into 2009....South Africa’s political and business elites seemed to languish in a state of denial and were broadcasting predictions of another year of positive economic growth, even as credit markets around the world asphyxiated and global demand dissolved” (Marais, 2010).

By the end of 2009 and early 2010, the façade was cracking. As will be shown later in this chapter, not only did employment decline, the considerable increase in the numbers of discouraged workers during that time suggests that the country was not immune from the international shock wave. The period was accompanied by an increase of more than 900 000 displaced workers (DPRU, 2009). The sharp fall in demand of its export products together with a fall in prices of key export commodities and dwindling foreign investment resulted in some concern (Chitiga et al. 2010). The particularly impressive performance of the post-apartheid economy, which averaged GDP growth of 3.3% from 1995 to 2003, declined in the second quarter of 2009 when the country was reporting its first recession in 17 years. This was the wake-up call, which exposed the underbelly of a vulnerable economic state buttressed by expanded public work projects. Since the last quarter of 2008, the declining demand for mining products and reduced manufacturing (which accounted for 16% of GDP and 14% of the workforce) had already showed signs of faltering. When the cumulative job losses in South Africa amounted to more than a million by the third quarter of 2009 (DPRU, 2009; ILO, 2010), some serious attention had to be paid. It was not surprising that by the close of 2009, the official prognosis was that the country was in recession. Even at this point, there was some downplaying of the challenge by some analysts. Verick (2010, p. 16) for instance suggests that "...the impact of the global financial crisis on the South African labor market is more evident in terms of rising discouragement, rather than a surge in official unemployment". This ignores the particularly salient fact that 'discouraged' workers, although not reported as officially unemployed, are indeed not employed. Indeed, the difference between broad (31.2%) and narrow (24.5%) unemployment at the height of the recession (between the second quarter of 2008 and the third quarter of 2009) reflects an increase in discouraged workers. The rise in the number of discouraged individuals, from 1.08 million in the second quarter of 2008 to 1.63 million in the third quarter of 2009 (*ibid*) provides telling evidence of considerable increase in unemployment not reflected in official figures, and thus significantly understated. There had clearly been a hemorrhaging of employment during the period which could simply not be ignored. The recession has unsurprisingly expanded the already widening disparity between 'haves' and 'have nots' outlined in the previous section. This inequity, associated as it was with differences in race, gender, age and level of education, was considerably affected. Table 1 shows the impact of the recession on key labour market variables in the period 2008 and 2009.

If unemployment was serious before the recession, the post-recession situation was especially disturbing. The impact on key equity indicators was particularly serious. African unemployment (using the broad definition) increased by 2.9% (from 31.9% to 34.8%) as compared to other racial categories. Male unemployment (3.6%) increased more than females (0.8%), and youth unemployment was significantly increased, by 6.1% in the age-group 15-24 and by 2.3% over 25. Those who completed matric (the school leaving examination) were more affected (3%) than those without (1.9%) suggesting the possibility of cutbacks affecting this group that were in all likelihood paid more highly. This meant that an already bad situation was worsened. In 2008, unemployment for 'Africans' (at 31.9%) was higher by almost 10 percentage points than other groups (Coloured at 21.1%). Female unemployment (32.9%) was slightly higher than their male counterparts (31.9%) and alarmingly, half of the youth were unemployed, at 50.3% in terms of the broad definition and 44.5% in terms of the narrow, with those between 15-24 (49.9%) higher than those above 24 (30.7%). Equity measures that were already under stress were therefore not assisted by the recession.

Table 1. Employment indicators in recession (2008 and 2009)

		2008 (Qtr 2)	2009 (Qtr 2)	Difference	Comment
Unemployment (Overall)	Broad Unemployment (%)	27.1	31.2**	4.1	Increase in absolute numbers of unemployed from 5.12 million in 2008 (Qtr 2) to 5.58 in 2009 (Qtr 2) (increase of 0.46 million). Also an Increase in discouraged workers from 1.1 million (2nd Qtr 2008) to 1,7 million (4th Qtr 2009)***
	Narrow Unemployment (%)	22.7	24.5**	1.8	Job losses: Manufacturing 149300/Retail Trade (223300)/Agriculture, Forestry and fishing (101100)
Unemployment (broad) by Race	African	31.9	34.8	2.9	Young african males with some education hardest hit
	Coloured	21.1	21.2	0.1	
	Indian	13.1	13.8	0.7	
	White	4.9	5.2	0.3	
Unemployment by Gender	Males	22.9	26.5	3.6	
	Females	31.9	32.7	0.8	
Unemployment by Age	Youth Employment (wide)	50.3	57.3**	7	Firms retrenching younger/inexperienced workers and not hiring new job-seekers
	youth unemployment (narrow)	44.5	48.4**	3.9	
	Age (15-24) (overall)	49.9	56	6.1	95% African/increase by > 200 000 (increased by 377 000 (3rd qtr 2009).
	Age (25-34) (overall)	30.7	33	2.3	youth unemployment serious challenge > no impact over 36
Unemployment by educational level	Completed matric	26.9	29.9	3	
	Grades 9-11	35.4	37.3	1.9	

Source: Statistics SA Quarter Labour Force Survey (Q2 2008 and Q2 2009) *Includes discouraged workers, ** Third Qtr 2009; ***ILO(2010).

Equity Implications

Slow growth due to the recession has perpetuated the skewed racial profile of the South African labour market. Table 2 reflects a snapshot of occupational level by race¹. As the footnote to the table points out, the data is premised on a broad illustrative reflection of the occupational profile of the sector, which is reflective of the key characteristics of the sector by race, rather than definitive statistics.

Clearly this picture complements the view of a racialized labor force. White males still ‘dominate the top echelons of workplaces’; are given recruitment and promotions and benefit the most from training and development opportunities (Nkeli, 2010). It reinforces the view of the Commission for Employment Equity that Whites are, “...continuing to be recruited and promoted in the private sector and are likely to continue to benefit from training and development opportunities” (Nkeli, 2010). Thus despite the range of equity (RSA, 1998b), and skills development legislation (RSA, 1998b), there is still considerable inequality and thus the urgent need for a coordinated response at all levels.

¹ In South Africa, the racial categories defined under apartheid are still used by national government to enable redress and serve as a mechanism for tracking progress towards post-apartheid societal equity. My use of these categorizations attempts to respond to the national imperative.

Table 2. Occupational skills profile of manufacturing employment, by race, 2008-2010 (%)²

	African		Coloured		Indian		White		Total
	2008	2010	2008	2010	2008	2010	2008	2010	
Managers	14	15.6	9.7	7.2	12.5	9.1	63.8	68.2	100
Professionals	25.7	44.8	16.1	9	10.9	10.6	47.3	35.6	100
Technicians	39.7	48.4	22.2	19.9	8	5.9	30.1	25.8	100
Clerks	39.5	38.5	22.7	20.3	11.7	10.8	26	30.4	100
Service and Sales	64.3	56.4	9.5	18.1	3.7	6.4	22.5	19	100
Crafts	68.8	68.3	17.1	17.5	3.4	3.3	10.8	10.8	100

Source: Statistics South Africa (2008, 2010): (StasSA, 2008) (StatsSA, 2009) Quarter Labour Force Survey (Q2 2008 and Q2 2009) *Includes discouraged workers, ** Third Qtr 2009; *** (ILO, 2010).

NATIONAL AND FIRM-LEVEL RESPONSES TO THE CRISIS: SKILLS AND ITS IMPACTS

South Africa's national response to the recession has been particularly counterintuitive to the broad developmental challenges to which it is subject. The first section identifies the key national initiatives that have been employed to respond to the immediate and medium term impacts of the recession, while the second identifies the way in which private sector companies have understood and responded to the most deleterious effects of the recession as it took hold.

National-Level Responses

Government's official response to the recession was contained in a tripartite agreement between government, business, and labour reached on 19th February 2009. Early in December 2008, the then President Motlanthe called a special "Joint Economic Working Group" to discuss the crisis and the response. Social partners agreed to develop a joint response and report back to the President. This report provided the substantial basis for responding to the crisis. The framework agreement reached after intensive negotiation incorporated the following principles:

- Protection of the vulnerable - the working poor and the poor
- Activities should support strengthening the capacity for future growth
- Agreement that bold counter-cyclical action is needed
- Continuing high levels of public investment to counter cycle

² Disaggregating manufacturing by occupation and race creates such small cells that the estimates are liable to be less than accurate to draw a meaningful trend analysis. For example, the 2008 data has only 76 professionals in manufacturing (unweighted), with only 19 African. The 2010 data has 69 observations for professionals of which 27 are African. Anything fewer than 100 observations is problematic, so the conclusions that can be drawn are only illustrative.

- Interventions must be timely, tailored and targeted (Hirsch, 2010)

As a social partnership response agreed to by labor, business and government, there was agreement around the following core principles: financing growth and investment; addressing distressed sectors; avoiding retrenchment and managing it; and addressing the social impact of recession. Significant infrastructural funding was also committed to mitigate the more serious impacts of the crisis. Infrastructural spending is scheduled to reach ZAR787 billion (circa US\$1 billion) over the period from 2009-2011 (part of which was already committed as result of the infrastructural requirements of the FIFA World Cup), and another ZAR390 billion (US\$8.8 billion) in capital spending by state-owned enterprises (Treasury, 2010). In addition, the consequent social spending commitment reflects a degree of seriousness to deal directly with the impacts of the crisis. A budget of ZAR24.8 billion (US\$3 billion) was allocated to health and education and another ZAR4.1 million (US\$0.5 million) for infrastructure. Serious attention was given to the social impacts of the crisis. Importantly, education spending was intended to increase over the next few years, from ZAR127 billion (circa US\$16 billion) in 2008/9 to ZAR169.7 billion (US\$21 billion approx) in 2011/2, an annualized growth of 10% in the period 2008-12 (*ibid*).

The core social protection element of the Government response to the recession was contained in a 'Training Layoff Scheme' (TLS). It was primarily designed to prevent retrenchments (layoffs) resulting from the crisis, by enabling (larger) companies to stave off some employee costs, while still retaining them in anticipation that the worst impacts of the recession could pass. It was, therefore, an attempt to 'delay' the impacts of the recession by reducing the employment costs. A government subsidy designed to enable companies to skill rather than lay-off workers seemed like a valuable strategy to 'manage' retrenchment. By assisting companies to deal responsibly with retrenchments that appeared inevitable, the TLS was designed to prevent large-scale retrenchments in an effort to, "...keep employees working during the economic downturn and re-skill them as an investment for the future economic recovery' (NEDLAC, 2009). Companies and unions would need to agree on the need for this measure in line with company conditions. Finance for this initiative would come from the national skills levy system - the National Skills Fund (ZAR1.2 billion – US\$150 million) and Sector Education and Training Authorities (SETAs) (ZAR500 million – US\$63 million). Only companies that 'were negatively affected by the economic downturn' were supported, with the assumption that if they were not supported, they would be 'forced' to retrench staff. The plan was, therefore, designed to assist staff in the lower echelons of the organization.

Companies were required to prove firstly that their profitability was impacted by the recession, and secondly that if they were not assisted, they would be 'forced' to retrench. While the intent of this program was based on principles designed to reduce the most negative impacts of recession (i.e., joblessness) on larger companies and their employees, it left the rationale for company-level job-shedding intact. It was assumed that retrenching workers was *fait accompli*. It also by default legitimated the view, perhaps an inevitable symptom of business process, that the only way cost-saving could be effected was by shedding personnel at the lower levels of the organization. Even if companies did not see this as a meaningful response, the incentive scheme appeared on the surface to encourage this and provided the rationale for companies to secure funds for this purpose. Thus, while the strategy was well

meaning in its intent to cushion the impact of the recession on those most vulnerable, it reinforced an unintended and unwelcome practice of retrenchment of the most vulnerable.

By its design, therefore, the program did not require proof that job-shedding would be the most effective response to the recession. It was assumed that it was. With respect to other cost-saving and/or profit inducing mechanisms, the reality of seeing lower level vulnerable workers as the most likely cost-saving component appears less than ingenious. The labor union's agreement was secured as they felt the need to reduce the impact of the worst effects of the recession on their membership, which was already being felt by increased retrenchment of their members.

The TLS also perversely (and perhaps tacitly) allowed the entity to leave the staffing of the upper level of the organization intact. This component was most likely to be better paid, arguably better skilled, and the component most in need of transformation. The TLS strategy clearly had potential to hold company recruitment practices accountable in their quest for assistance. It could, therefore, have been better utilized to effect transformation of the workforce of those companies requiring assistance. Instead of the potential to review the nature of structural employment, it left the racially defined employment structure intact and unquestioned. The possibility for company-level transformation offered by the TLS was, therefore, muted. Importantly, in its attempt to respond to the immediate symptoms (i.e., the 'economics' of the crisis) via its 'short-termist' perspective, it not only reinforced assumptions about how to deal with the crisis, but also inadvertently left the racial structure unscathed.

The potential for national development equity and transformational impacts was therefore compromised by the TLS. More saddening was that the implementation of this initiative was considerably less than anticipated. By October 2010, only 6514 workers had been assisted in this initiative. Much of the funds were unspent (ZAR 2.5 billion of ZAR 2.9 billion) (Hirsch, 2010). By October 2010, only ZAR400 million (US\$50 million) was spent. Whether this was a result of bureaucratic processes that needed to be loosened or capacity to unlock funds, it is clear that that a golden opportunity to induce transformation was lost.

Where assistance was provided, government effectively provided support to businesses that were, in many instances, likely to have a skewed workforce profile. The potential to insert a transformative agenda to enable workplace equity in the private sector was therefore lost. The Training Layoff Scheme (TLS) had potential to demand some commitment to equity, as an absolute pre-condition for state assistance. As it was, little attention was paid to the skewed racial structure of the corporations that were likely to be assisted.

Firm-Level Responses

The information derived at company level was gleaned from two exclusive processes. First, a quantitative survey of companies undertaken in 2009 and second, a qualitative survey of 32 key individuals, who were asked to report on skills acquisition practices during recessionary times. They provide important information about the way in which companies understand skills development and recruitment. They were both undertaken prior to the national initiatives being implemented.

Table 3. Company response to impact of recession on key skills development categories: Supply, demand and training spend (%)

	To some/large Extent	Not at all/really	Total
Supply of skilled trade worker or artisan	43	57	100
Demand in the need for skilled artisan/trade workers	60	40	100
Training spent in the organisation	59	41	100

Source: SETA (2010) Employability Survey.

The qualitative survey showed that there was uneven impact on various economic sectors, based on the anticipated buoyancy of the market, and primarily based on perceived government response. For those companies in sectors expecting government intervention and bailout, the recession served as an enabling mechanism for skills growth. While most companies reported that they needed to cut back on skills, some froze training budgets completely. At larger companies, most training was dramatically reduced and enrolment in skills programs virtually halved. In the Automotive Sector, however, artisan training and operator-level training in support of new vehicle model launches and new plant establishment generally continued in anticipation of a renewed government incentive support mechanism. Small- and medium-sized firms reported that they sustained this throughout the crisis. In addition, legislated training such as safety, health and environment was not compromised at every level. The legal requirement for this training form ensures that this continues.

Information from companies also reflected strongly the expectation of companies for effective and assertive state intervention. Most companies surveyed were particularly critical of the government assistance responses referring to them as being too little, too late. Expectations of state intervention in times of recession, while not unusual in conventional contexts, is perhaps heightened and more pronounced in South Africa, where skills levies are used to encourage firm-level skills development.

The quantitative survey provided important data about the nature of skills supply in times of recession and complemented information derived from the qualitative interviews. It showed that the recession has had little positive impact on the nature and quantity of skills supply/availability, as Table 3 indicates.

Importantly, the negative impact on training spend in companies appears to be increasingly dire. Of the companies surveyed, 60% indicated that the recession has had negative impact on training in the organization, with 59% of companies indicating that training spend declined to a significant extent.

Survey data indicated the national trend of a racialized labor force. Whites continue to dominate the top echelons and this view has been reinforced by information undertaken in another survey as depicted in Figure 2 below.

Whites dominate the upper echelons of the organization in the managerial/professional category (73%) and 'Sales and Service (50%) sectors. A little under half (47%) of those employed considered to be in the 'skilled' category were white, suggesting dominant employment/recruitment practice and/or the dearth of black skill in this category.

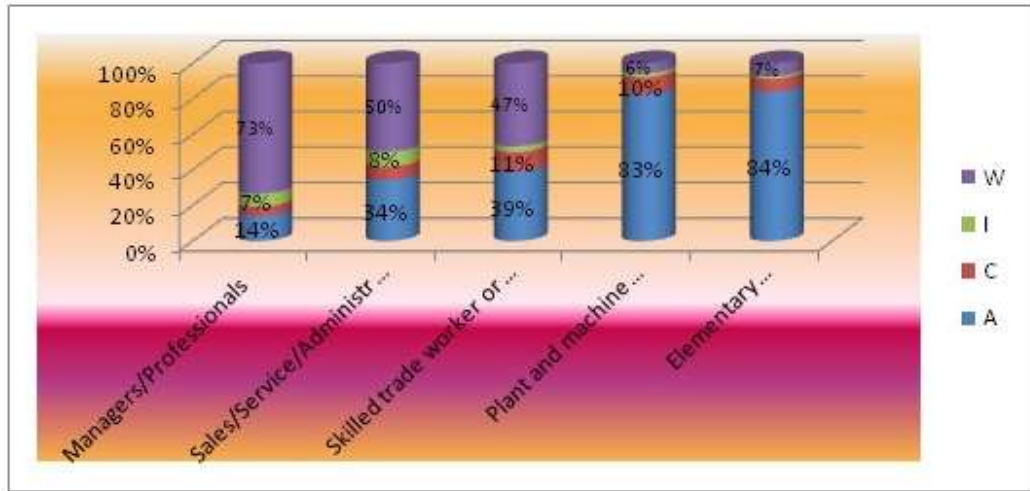


Figure 2. Demographic profile of labor force by race. Source: SETA (2010) Employability survey.

Clearly, the employment prospects for blacks appear bleak. An overview of recruitment practices in the survey suggests the lack of any transformational impetus. Although business expansion was muted during the crisis, there was evidence that recruitment was still robust during the recession. Most companies reported having to recruit in the skilled components of the organization. More than a third of companies surveyed (36%) recruited at the 'skilled worker' or 'artisan' category, followed by the 'plant and machine operator' (28%) level, and few companies (16%) recruiting at the 'elementary worker' level. Very few firms recruited at the higher echelons such as management (9%) and sales and service personnel (12%), suggesting that the possibility for change in the upper structure is minimized.

Recruitment trends also show a distinct lack of workforce transformation in these times. Table 4 shows the racialized structure of recruitment practices. 'Whites' have been recruited at the upper echelons of the organization (manager, sales and skilled worker levels), and "blacks" at lower levels, 'elementary' and 'plant level operator' level as table 4 shows. Thus, the possibilities for demographic transformation have been muted in recessionary times.

Recruitment during recession is also associated with considerable poaching of experienced personnel from other companies, ensuring that those hired with some experience are able to 'hit the ground running' so as to incur minimal production downtime required for technical and workplace familiarity.

More than two-thirds of companies reported that they recruited from other companies (76%), as opposed to a little over a fifth (24%) recruiting newly trained graduates from colleges and universities. This unsurprising propensity to reduce the 'burden' of training in this time was clearly evident.

The reality of firm-level response was, on the one hand, to cut back on existing training and, on the other, to recruit from an existing pool of skilled workers. The impact on firm-level transformation was, therefore, muted as companies used skills already in the country.

Table 4. Recruitment by occupation and race

	African	Coloured	Indian	White	
Managers/Professionals	33%	5%	7%	55%	100%
Sales/Service/Administration and secretaries	19%	8%	8%	65%	100%
Skilled trade worker or artisan	27%	7%	6%	60%	100%
Plant and machine operators	60%	12%	6%	23%	100%
Elementary workers/Labourers	85%	5%	1%	9%	100%

Source: SETA (2010) Employability survey.

CONCLUSION

While international attention has been placed on responding to the economy, there are examples of contexts in which skills has loomed large in responding to the current crisis. This is indeed to be welcomed. However, in South Africa, where an economic response targeting sustainability of larger entities has been the focus of the recessionary response, the immediate gains of economic sustainability appeared to have ignored the larger equity imperative bedeviling the country. The crisis provided a golden opportunity to engage workplace equity challenges which are still evident in the private sector. Clearly an examination of the equity and transformation impacts and responses suggests that it has been sacrificed and the national equity project compromised in an effort to reap short term gains. The challenge for transformation of large workplaces in the attempt to achieve a more diverse employee profile of the private sector in South Africa still remains.

The examination of national responses to the crisis, complemented by firm-level data of responses, suggests that the official South African response to the recession impacted marginally on key national development challenges. The ‘Training Layoff Scheme’ introduced by the national government as a response to the crisis to stave off retrenchment provided little consolation to the broader equity challenge. Importantly, it provided the rationale for larger companies to target labor costs of those most vulnerable as a cost-reducing measure, while simultaneously leaving the upper echelons of the organization intact, the area most in need of transformation. The real issue of changing the workforce profile was therefore left for the post-recession era. Importantly, it also left smaller business entities, considered to be a crucial component of the response to the employment challenge, outside of the loop of the assistance plan.

Recruitment practices of companies during this time suggest that transformation imperatives have been significantly undermined. Most importantly, recruitment, where it was occurring, was racially defined, with companies reinforcing their racially defined upper structure by recruiting from an already dwindling pool of white candidates. This is not unsurprising in a context when firms seek what they perceive as the most efficient and effective skills capable of responding to crisis effectively in challenging economic times.

The chapter has argued that the call for state support presented a ‘golden opportunity’ for the establishment of more equitable workplaces. While it is accepted that the possibility for

voluntary equity achievements is much more elusive than anticipated, the call for state support to larger private sector entities provided the opportunity for ensuring equity outcomes. The skills development incentives program could have been a powerful means by which employment equity could have been effectively advanced. The various reports by the equity commission lamenting the lack of transformation in the workplace suggest that all means by the state need to be utilized to achieve equitable workplaces. Perhaps the real challenge is to nudge business to take on the transformation agenda by means that are economically defensible, a regrettable situation, since the transformation imperative has an intrinsically legitimate and plausible business incentive component.

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Chapter 22

FOREWORD ADVANCES AND CHALLENGES IN STROKE: HOW ARE MODERN TECHNIQUES CHANGING OUR UNDERSTANDING OF STROKE?

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INTRODUCTION

Stroke has a sudden and sometimes devastating impact on both patients and their families. According to the WHO definition, stroke is a cerebrovascular accident caused by “the interruption of the blood supply to the brain (...). This cuts off the supply of oxygen and nutrients, causing damage to the brain tissue.” In reality, a very complex world of numerous biochemical, molecular, and pathological processes is hidden behind this simple definition. Although stroke is most common in older people, it can occur in individuals of any age, including young adults and children. Fortunately, the prospects of preventing and treating stroke are far better nowadays than they were just few decades ago. Today we know a lot more in terms of pathogenesis, risk factors and genetics. Moreover, treatment of stroke has improved dramatically, reducing mortality and the likelihood of long-term disability.

However, many challenges still await physicians and researchers in prevention, understanding of pathogenesis and optimizing treatment of this condition. Stroke remains an emergency for our society; all over the world about 15 million people suffer from cerebrovascular diseases, stroke being the most frequent life-threatening neurological disease and the third cause of death and disability.

With this book we will accompany the readers through the complexity of stroke pathogenesis, from the molecular basis to the most recent advances in term of pathogenesis and treatment. “Advances and challenges in stroke” aims to examine some aspects of interest in the field of cerebrovascular disease, such as the etiology of juvenile and cryptogenic stroke,

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the relationship between heart and brain in the pathogenesis of brain injury, the telemedicine state of the art in reducing door to needle and decision-making times as well as the contribution of modern neuroimaging techniques and of surgical or endovascular treatment in the hyperacute stroke phase.

It has been a great experience to serve as the Guest Editor for this monography. I hope this publication offers a new momentum in advancing the science of stroke. I would like to express my special gratitude to the Nova Publishers for offering us this unique opportunity. I sincerely thank my Director and Assistant Editor of this book, Professor Ubaldo Bonuccelli, for giving me the chance to follow this exciting project, and for having been actively involved in the designing of this challenging book. Moreover, I deeply thank all the authors of this issue for their timely and invaluable contributions to this monography. Finally, I have to thank my young fellows for their daily effort in both managing stroke patients and research activity: Nicola Giannini, Miriam Maccarrone, Vincenzo Montano, Leonardo Ulivi.

Enjoy the reading!

Chapter 23

MONOGENIC DISEASES ASSOCIATED WITH STROKE

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ABSTRACT

Ischemic stroke is a complex disorder resulting from the interplay of genetics and environment. In some instances (up to 5% of cases, especially in young adults) stroke is the direct result of a monogenic disease. Among the monogenic causes of stroke, the diseases that most frequently encounter in the adult general neurological practice are CADASIL, Fabry disease and mitochondrial disorders. In many instances, brain magnetic imaging and clinical data may help in the molecular screening. The identification of a genetic cause is important for appropriate counseling and in order to start a correct therapy, where available. Here we review the single-gene causes of ischemic stroke, with special regard to the associated features which can be helpful in the diagnostic process.

Keywords: stroke, genetics, MELAS, CADASIL, Fabry, DNA

INTRODUCTION

Stroke is the most common life-threatening neurological disease, the third largest cause of death and disability in the developed countries and a leading factor in age-related cognitive decline and dementia [1]. Indeed, stroke is considered a multifactorial disorder, resulting from the interaction between many genes and environmental factors. Conventional vascular risk factors like hypertension, diabetes mellitus, obesity, cigarette smoking and atrial fibrillation

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account for about 50% of stroke risk; however, these factors do not entirely account for the occurrence of stroke in unexposed populations and also fail to explain the incidence of stroke in select individuals within a population that is uniformly exposed to environmental risk factors [2]. Some of this phenotypic variability has been attributed to genetic differences, with familial patterns of inheritance also providing support.

It is likely that multiple genes are involved in stroke pathogenesis, severity and response to treatment (not dissimilar to other complex polygenic diseases) [3]. Genome wide association studies (GWAS) on a wide range of candidate pathways, such as the haemostatic and inflammatory system, homocysteine metabolism, and the renin-angiotensin-aldosterone system, suggest a weak but significant effect for a large number of single nucleotide polymorphisms (SNPs) [3, 4]. This is a promising approach for the identification of novel biological mechanisms that underlie the development of cerebrovascular disease.

In some instances (up to 5% of stroke cases, especially in young adults) stroke is the direct result of a monogenic disease [5]. The majority of these monogenic disorders begins at young age, and frequently affects multiple organs and tissues. It is important to consider inherited syndromes in the differential diagnosis particularly in cryptogenic stroke and in patients with a family history of stroke. The identification of a genetic cause is important for appropriate counseling and in order to start a correct therapy, where available. Finally, unraveling the mechanisms of disease responsible for monogenic stroke disorders may contribute to future understanding of the pathophysiology of sporadic stroke syndromes.

Here we review the single-gene causes of ischemic stroke, with special regard to the associated features which can be helpful in the diagnostic workup.

SMALL-VESSEL DISEASES (SVD)

Cerebral SVD refers to pathological processes that affect the structure or function of small vessels on the surface and within the brain, predisposing to ischemic and, in some instances, hemorrhagic stroke and diffuse white matter disease [6]. Neuropathology is usually characterized by thickening of the arterial media of cerebral small vessels with encroachment on the arterial lumens (typically due to the deposition of material or hypertrophy of smooth muscle/other connective tissue elements), multiple small deep lacunar infarcts and degenerative abnormalities in the cerebral white matter. Clinically, SVD can progress silently for many years before becoming clinically evident [7].

Currently, there are no specific proven treatments for SVD (with the remarkable exception of Fabry disease), and therapeutic options for secondary prevention are particularly limited compared with those for other common causes of stroke; thus, treatment is entirely symptomatic.

Hereditary forms of cerebral amyloid angiopathy (CAA) are not discussed here because they are much more frequently associated with lobar hemorrhages, microbleeds, cortical petechiae and subcortical hemorrhages rather than ischemic strokes; dementia and diffuse white matter damage are very common in these conditions [8]. Amyloid precursor protein (*APP*) is the gene more frequently involved in most familial forms of CAA [9].

Cerebral Autosomal Dominant (and Recessive) Arteriopathy with Subcortical Infarcts and Leukoencephalopathy

CADASIL is an autosomal dominant (AD) disorder caused by dominant mutations in the *NOTCH3* gene on chromosome 19p13.2-p13.1 [3]. *NOTCH3* encodes a large transmembrane protein receptor located on cell surface expressed in vascular smooth-muscle cells and pericytes, essential for arterial differentiation and development [10]. CADASIL usually presents in youth and adulthood with migraine, transient ischemic attacks (TIA) or strokes, psychiatric disorders and cognitive impairment. It is now recognized as the most common cause of inherited stroke and vascular cognitive impairment in adults [11]. Interestingly, Schmidt H. et al. have shown that 4 single nucleotide polymorphisms (SNPs) in the *NOTCH3* gene are significantly associated with an increased risk and progression of age-related white matter lesions in hypertensives, suggesting that genetic variations in *NOTCH3* also play a role in age-related SVD [12]. Transient ischemic attacks and ischemic stroke in CADASIL occur at a mean age of 47 years (age range 20-70 years), in most cases without conventional vascular risk factors. Ischemic events are subcortical and present in most individuals as lacunar syndromes [13], due to microangiopathy accompanied by the presence of granular osmiophilic material (GOM) of 0.2-1 μm in size in the arterial walls of brain. GOM form deposits in the smooth muscle cells of the media long penetrating arteries supplying subcortical white matter leading to luminal stenosis and reduction in cerebral blood flow [14]. Though changes appear to be most severe in cerebral WM, they may be also observed in the media of dermal, peripheral nerve and muscle arteries; therefore, if molecular genetic testing is not conclusive, the diagnosis could be established by detection of GOM by electron microscopy and immunohistochemistry of a skin or skeletal muscle biopsy [15]. CADASIL should be considered in patients with a history of migraine (which is often the first symptom [13]), especially with atypical prolonged aura, subcortical stepwise dementia manifesting initially with executive dysfunction, recurrent TIA/strokes (before age of 60-yrs), and psychiatric symptoms [3]. Apathy and mood disturbances are typical features. Brain magnetic resonance imaging (MRI) shows white matter hyperintensities typically in the temporal poles, external capsules, periventricular and deep white matter with the highest lesion load in the frontal lobe, lacunar infarcts, dilated perivascular and cerebral microbleeds (located predominantly in the thalamus) [16]. The genetic background may play a role in modulating the overall white matter lesions burden in CADASIL explaining the phenotypic variability between individuals as demonstrated by Gesierich et al. who found a significant association of APO ϵ 2 allele with increased white matter hyperintensities volume [17]. Cardiovascular risk factors may also worsen the phenotype of CADASIL and should be treated aggressively [13]. Given this, prevention of ischemic attacks is commonly based on treatment of vascular risk factors and antiplatelet drugs rather than anticoagulants because of the increased risk of cerebral hemorrhage [11]. However, the benefits of platelet antiaggregates for CADASIL have not been established to date [11].

Cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) is similar to CADASIL but with earlier onset and systemic symptoms. CARASIL is due to mutations in the *HTRA1* gene localized on 10q26.13, encoding a serine protease belonging to the HTRA protein family that represses signaling mediated by the transforming growth factor- β (TGF β) [18]. The disease is predominant in males, with a male:female ratio of 3:1; to date most patients described in literature (about 50)

have been reported in Japan and China, though it could be an underdiagnosed condition [3]. Clinically frequent initial symptoms are gait disturbance with spasticity and pyramidal signs, and then the clinical pictures evolve with early (between 20 and 50 years) slowly progressive dementia and mood changes [19]. Differently from CADASIL, CARASIL has a multisystem involvement characterized by alopecia, arthropathy, low back pain, spondylosis deformans and disc herniation [20]. Typical neuropathological features are arteriosclerosis associated with intimal thickening and dense collagen fibers, loss of vascular smooth muscle cells, and hyaline degeneration of the media of cerebral small arteries of white matter and basal ganglia resulting in luminal stenosis; unlike CADASIL, GOM within the vascular media is never observed and skin biopsy is not useful for diagnosis [21]. Brain MRI does not allow to differentiate CARASIL from CADASIL with certainty considering that this two SVD share the same pattern of white matter hyperintensity on T2-weighted or fluid-attenuation inversion recovery (FLAIR) images in periventricular and subcortical white matter, anterior temporal lobe, cerebellum, brain stem, external capsule and lacunar infarctions. A typical MRI finding in advanced stage of CARASIL is an arc-shaped hyperintense lesion from the pons to the middle cerebellar peduncles, designated as “the arc sign” by Nozaki et al. [22]. Spinal MRI demonstrates spondylosis deformans and disk degeneration in cervical and lumbar tract [18]. Inhibition of accelerated TGF- β signaling may be in the future an interesting therapeutic approach for CARASIL. Recently, Verdura et al., using a whole exome sequencing approach, found a heterozygous *HTRA1* pathogenic mutation in a family with AD-SVD of unknown etiology and in 10 of 201 additional unrelated and affected probands; they also found a significant difference in the number of likely deleterious variants in cases compared to controls, suggesting a role of *HTRA1* gene in sporadic SVD of unknown aetiology [23].

Fabry Disease

Fabry disease is a rare, X-linked, inherited disorder of glycosphingolipid metabolism [24]. It is a lysosomal storage disorder with progressive multiorgan involvement, caused by a partial or total deficit of α -galactosidase A (α -GalA) enzyme. α -GalA is encoded by *GLA* gene, located on the long arm of chromosome X (Xq22). Clinical manifestations of Fabry disease result from the accumulation of glycosphingolipids, particularly globotriaosylceramid (also called lyso-Gb3) and galactosylceramide in many tissues and cell types, including endothelial cells, kidney, heart and neurons. Tissue damage is thought to be at least partly due to poor perfusion [25].

Progressive involvement of kidney, heart, and cerebral vessels leads to renal failure, hypertrophic cardiomyopathy, and strokes in adults represent major causes of disease-related morbidity [25]. Gastrointestinal symptoms, hypohidrosis, angiokeratomas and corneal opacities (cornea verticillata) are also common and may aid in the diagnostic approach [25]. Neurological presentations of Fabry disease include stroke episodes, painful neuropathy (small fiber neuropathy), and aggressive clinical forms with central multifocal, relapsing, and progressively debilitating symptoms [26, 27]. Atypical neurological presentations (e.g., transient global amnesia [TGA]-like episodes) are rare but possible [28]. Women may remain free of serious complications, but most are symptomatic and can have cardiac disease and stroke. From this point of view, Fabry disease can be considered a dominant X-linked disease

with incomplete penetrance and variable expressivity (higher in males). Variation in clinical manifestations in heterozygous females is attributed to random X-chromosome inactivation.

Fabry disease is a heterogeneous condition, and the diagnosis may require a multidisciplinary approach [29]. Along with progressive renal, cardiac and sensorineural audiological involvement, stroke is a major and often life-threatening burden of the disease; cerebral vasculopathy occurs commonly and leads to ischemic cerebrovascular events at an early age [30]. The posterior circulation seems especially susceptible, and Fabry disease should be considered in the differential diagnosis of all young cryptogenic stroke cases [30]. The most prominent MRI findings are severe progressive white-matter lesions [30]. The “pulvinar sign” (T1-weighted hyperintensity within the pulvinar), when present [26], is also suggestive.

Enzyme replacement therapy (ERT) is now available, making the diagnosis of this disease essential. Early initiation of treatment is needed for improvement in major affected organs with decreased cardiac mass and stabilisation of kidney function, and improvement in neuropathic pain, sweating, gastrointestinal symptoms, hearing loss, and pulmonary symptoms [31]. Treatment of individual symptoms in addition to ERT is needed for many patients, especially those presenting with organ dysfunction.

Other Rare Autosomal Dominant Small-Vessel Diseases

COL4A1 is a gene encoding the type collagen IV $\alpha 1$ chain that causes a spectrum of cerebrovascular phenotypes with overlapping features which comprises autosomal dominant type 1 porencephaly, brain small-vessel disease with hemorrhage, brain small-vessel disease with Axenfeld-Rieger anomaly and hereditary angiopathy with nephropathy, aneurysms, and muscle cramps (HANAC) syndrome [32, 33]. The presence of a *COL4A1* mutation compromises the vascular basement membrane and weakens the vessel, predisposing to rupture. Juvenile stroke is the core clinical features of *COL4A1* small vessels disease, either ischemic (usually lacunar) and hemorrhagic; recurrent intracerebral hemorrhages may occur in non-hypertensive adults who are younger than age 50 years [34]. Other neurological features are infantile hemiparesis, seizures, migraine with visual or sensory aura, intracranial aneurysms, developmental delay, cognitive impairment and dementia. Systemic features include cataracts, retinal hemorrhages, nephropathy with hematuria and bilateral renal cysts and muscle cramps with elevated creatine kinase levels. Brain MRI shows diffuse leukoencephalopathy with deep white matter involvement of posterior periventricular areas, subcortical infarcts, microbleeds and intracranial cerebral aneurysms [34].

Autosomal dominant retinal vasculopathy with cerebral leucodystrophy (RVCL) is a microvascular endotheliopathy presenting with visual loss, stroke and dementia, with onset in the fourth or fifth decade and sometimes a fast clinical course (5 years or less). Cerebral small vessels in RVCL show fibrinoid vascular necrosis, thickened hyalinized vessels with unusual multilaminated basement membranes and with white matter ischemia [35]. RVCL is caused by mutations in *TREX1* (three-prime repair exonuclease 1) gene [36]. The retinopathy is characterized by retinal hemorrhages, macular edema and neovascularization of the optic disc responsive to bevacizumab [37]. Neurological manifestations are TIAs and strokes, progressive cognitive involvement, headache and psychiatric symptoms. In about 50% of

patients, brain MRI demonstrates, in addition to white matter hyperintensities, an enhancing tumor-like lesion with cortical sparing that may resemble a primary CNS malignancy [38].

Homocystinuria

Homocystinuria encompasses a group of mostly autosomal recessive enzyme deficiencies causing very high plasma ($>100 \mu\text{mol/L}$) and urine concentrations of homocysteine [3]. The classic disease is usually caused by deficiency of cystathionine β -synthase (CBS), a pyridoxine (vitamin B6)-dependent enzyme. It should be suspected in patients with multiple organ involvement with infancy or early childhood onset, clinically characterized by developmental delay, cognitive impairment, severe myopia, Marfan-like skeletal abnormalities (i.e., excessive height and long limbs), osteoporosis and thromboembolic events [39]. The measurement of plasma and urine amino acids levels, the assay of CBS enzyme activity, or the molecular genetic testing of *CBS* can establish the diagnosis of classic homocystinuria in a proband. The disease must be distinguished from the milder ($15\text{--}100 \mu\text{mol/L}$) hyperhomocysteinaemia, which is a risk factor for stroke in the general population, generally associated with both deficient food intake of B6, B12 vitamins and folic acid and a common substitution, $677\text{C}\rightarrow\text{T}$, in the gene for methylenetetrahydrofolate reductase (*MTHFR*) [40]. Pathogenesis of stroke in homocystinuria includes early atherosclerosis, carotid intraluminal thrombosis, arterial dissection, small vessels disease and cardiac embolism [41]. Two phenotypic variants are identified, the B6-responsive (usually the milder) and the B6-non-responsive; the vitamin B6-responsiveness is determined by a pyridoxine challenge test; however, it can be predicted by the genotype [42]. Screening for subclinical hyperhomocysteinaemia in young adults with premature stroke is essential even in absence of classic phenotypic features of homocystinuria, as complications can be reduced [41].

STROKE-LIKE EPISODES

Stroke-like episodes (SLE) resemble ischemic stroke, but they do not usually have an arterial distribution, and histopathological studies don't find lesions of the major cerebral blood vessels.

Mitochondrial Diseases

The "MELAS" acronym (Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes) includes patients who experience stroke-like episodes, with an histological (i.e., ragged red and/or COX-negative fibers in skeletal muscle), biochemical and/or molecular evidence of mitochondrial disease. Lactic acidosis is frequently associated. Adjunctive features may be generalized seizures and cognitive impairment. Typical "mitochondrial red flags" are muscle weakness, exercise intolerance, eyelid ptosis, ophthalmoparesis, axonal multifocal neuropathy, sensorineural hearing loss, pigmentary retinopathy, optic neuropathy, diabetes mellitus, hypertrophic cardiomyopathy and Wolff-

Parkinson-White syndrome, migraine, short stature [43]. The commonest mutation associated with MELAS is the m.3243A > G in the mitochondrial DNA (mtDNA) gene encoding the mitochondrial tRNA leucine 1 (MT-TL1) [44]. However, other mtDNA mutations may cause SLE as well; for example, they can be encountered in the clinical spectrum of the m.8344A > G mutation [45]. Gene analysis for mtDNA can be performed in muscle samples and urinary sediment, because these analyses are less sensitive in blood cells [43]. If the m.3243A > G mutation is absent in the skeletal muscle, the entire mtDNA should be sequenced from the muscular DNA. Genetic studies on blood cells are more useful for nuclear DNA. On the other hand, some nuclear gene mutations (i.e., in *POLG* and *PEO1* genes, encoding respectively the mitochondrial polymerase gamma and the helicase “Twinkle”) can cause a MELAS-like syndrome [46, 47].

Among the affected patients, the most severe COX deficiency (associated with the highest proportion of mutated mtDNA) was observed in the leptomeningeal and cortical blood vessels' wall (in the whole brain), supporting the hypothesis of a vascular mitochondrial dysfunction in the pathogenesis [48]. There is consensus about the vasogenic oedema nature of the SLE lesions; unfortunately, evidences in treatment of SLEs come from single-case reports or small case series and no standardized therapy is available to date [49]. L-arginine is usually administered intravenously in a dosage of 0.4-0.5 g/kg, and may be not only effective for the SLE in the acute phase but also for concomitant refractory epilepsy; it should be switched to long-term oral supplementation after the acute phase [49]. SLE may also improve with corticosteroids, as observed in single cases [50]. Other molecules (i.e., creatine, coenzyme Q10, idebenone) have been suggested to be potentially beneficial in single case reports [50]. Until effective drugs will be available, the treatment of choice may be represented by the combination of L-arginine against the vasogenic oedema, with anti-epileptic drugs and supportive care when needed [51]. Drugs with potential mitochondrion-toxic actions (i.e., valproic acid, metformin, etc.) have been recently reviewed elsewhere [51] and should be avoided.

Familial Hemiplegic Migraine

Familial hemiplegic migraine (FHM) is an autosomal dominant classical migraine subtype that typically includes fully reversible weakness of half the body which can last for hours, days or weeks and other symptoms like visual disturbance (the most common), sensory loss or dysphasia [52]. Basilar migraine and cerebellar signs (ranging from nystagmus to progressive, usually late-onset, mild ataxia) are present in 40-50% of cases. Episodes of reversible coma, SLE, seizures, progressive ataxia, psychosis and severe intellectual disability are uncommon [53]. While FHM often have an earlier onset than typical migraine, the frequency of attacks tends to decrease with aging. So far, three genes are known to be associated with FHM: *CACNA1A* (FHM1), *ATP1A2* (FHM2), and *SCN1A* (FHM3). Mutations in the *CACNA1A* gene (encoding the alpha1A sub-unit of the voltage-gated calcium channels in neurons) are responsible for 50% of FHM cases [54]. Genotype-phenotype correlations have been described [54]. MRI does not show white matter abnormalities or sub-cortical strokes, but it may reveal various degrees of vermian cerebellar atrophy [53]. Treatment options include acetazolamide, migraine prophylaxis and antiepileptic drugs [55].

DISORDERS OF THE CONNECTIVE TISSUE

COL4A1-associated disease has been considered above (“Small-vessel diseases”).

Ischemic stroke is a well-known complication of several inheritable connective tissue disorders, which more frequently cause large-vessel diseases. Apart from those considered in the following paragraphs, many other hereditary connective tissue diseases may, rarely, present with stroke [56].

Marfan Syndrome

Marfan syndrome is an autosomal dominant disorder, caused by mutations in the fibrillin 1 (*FBN1*) gene, affecting the musculoskeletal and cardiovascular systems and the eye. The typical clinical features are myopia, displacement of the lens, bone overgrowth (disproportionately long extremities for the size of the trunk), joint laxity, scoliosis, *pectus carinatum* or *excavatum*, arachnodactyly and dilation or dissection of the ascending aorta [57]. Cerebrovascular complications of the disease include TIAs, ischemic strokes, subdural haematomas and cerebral aneurysms [58]. A cardiac source of embolism is the leading cause of ischemic stroke in Marfan syndrome, followed by cervical artery dissection [59].

Vascular Ehlers-Danlos Syndrome

Ehlers-Danlos syndrome type IV, the “vascular” type, is an autosomal dominant disorder, resulting from mutations in the collagen III gene (*COL3A1*), encoding for collagen type III. Vascular Ehlers-Danlos syndrome leads to severe fragility of connective tissues, predisposing arteria, uterus and intestine to ruptures, sometimes during surgical and radiological treatments. Other systemic clinical features include easy bruising, thin skin with observable veins, characteristic facial tracts (thin nose, lips and philtrum, small chin, large eyes), acrogeria, tendon or muscle ruptures and chronic joint subluxations or dislocations. Cerebrovascular events are common and include spontaneous cervical arterial dissection, intracranial aneurysms, subarachnoid hemorrhages, carotido-cavernous fistulae [60].

Pseudoxanthoma Elasticum

Ischaemic stroke can occur in the natural history of Pseudoxanthoma elasticum, an autosomal recessive (rarely dominant) disease due to *ABCC6* (ATP-binding cassette C6) gene mutations. Pseudoxanthoma elasticum is a systemic disorder that affects the elastic tissue of the skin, the eye, the cardiovascular and gastrointestinal systems. Individuals most commonly show skin changes (like increased elasticity, yellow-orange papular lesions), ocular abnormalities (retinal angioid streaks), gastrointestinal bleeding and hypertension. Degenerative changes of vascular wall, with various degrees of intima thickening due to a patchy proliferation of the fibroelastic components, cause arterial narrowing, increasing the

risk of ischemic stroke (large vessels or small vessels disease subtypes) and, sometimes, predisposing to cervical artery dissection [61].

OTHER MONOGENIC DISEASES

Hereditary prothrombotic disorders (e.g., Factor V Leiden mutation; protein C, protein S, antithrombin deficiencies) are more commonly associated with cerebral venous thrombosis and will not be discussed here.

Sickle Cell Disease

Hemoglobin S (HbS) results from a substitution of thymine for adenine in the beta chain gene of the protein. Sickle cell disease is due to a homozygous or a compound heterozygous state, the last with HbS occurring in combination with other hemoglobinopathies. The disorder is most prevalent in African or African American descent. The mutation causes polymerization or aggregation of deoxygenated hemoglobin within red blood cells. Patients commonly suffer from chronic compensated hemolytic anemia, mild jaundice, and intermittent vaso-occlusive crises (excruciating pain in the back, chest, and extremities). Vasculopathy in sickle cell disease has a complex pathophysiology, involving abnormal red blood cell adherence to the endothelium, oxidative stress, endothelial cell dysfunction, hypercoagulable state, chronic inflammation and vasomotor tone alterations, leading to nitric oxide depletion [62]. Stroke in sickle cell disease is mainly due to the large-artery vasculopathy of the proximal middle cerebral arteries and intracranial internal carotid arteries. Other features of central nervous system involvement are TIAs, hemorrhagic strokes (associated with aneurysms), silent ischemic lesions in the territory of penetrating arteries, seizures, spinal cord vascular events and Moyamoya-like syndrome that occur in about 25% of individuals by the age of 45 years [63]. Transcranial Doppler (TCD) ultrasound detects narrowed arterial segments and helps predict which patients are at higher risk of stroke [64, 65]. Transfusion programs with the goal of decreasing the HbS below 30% significantly reduce stroke risk in patients with abnormal TCD [66]. Other treatment options include hydroxyurea and, in some instances, bone marrow transplantation.

Hereditary Hemorrhagic Telangiectasia

Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disease, characterized by the presence of multiple arteriovenous malformations (AVMs) [67]. HHT is caused by the mutation in two genes involved in the TGF- β /BMP signaling, *ENG* (HHT1) and *ACVRL1* (previously named *ALK1*, HHT2), but recently HHT has been also associated to *SMAD4* (typically with concomitant juvenile polyposis) and *GDF2* [68]. Spontaneous and recurrent epistaxis from juvenile age, mucocutaneous telangiectases and gastrointestinal bleeding, usually after the age of 50 years, are the common manifestations of the disease. AVMs are found in many organs, including brain, lung, liver, pancreas and bowel.

Table 1. Adjunctive clinical features in juvenile stroke

	Nervous system	Cardiovascular system	Eye	Skin	Others
Fabry disease	Pain, hearing loss, white-matter lesions	Hypertrophic cardiomyopathy	Corneal opacities	Hypohidrosis, angiokeratomas	Renal failure, gastrointestinal symptoms
CADASIL	Migraine with aura, psychiatric disorders, dementia, leukoencephalopathy				
CARASIL	Leukoencephalopathy, cognitive involvement			Alopecia	Arthropathy, back pain, spondylosis
<i>COL4A1</i>	Infantile hemiparesis, seizures, visual loss, dystonia, hemorrhagic strokes, migraine, dementia, leukoencephalopathy				
<i>TREX1</i>	Cognitive involvement, headache, psychiatric symptoms, leukoencephalopathy		Retinal telangiectasias, microaneurysms and capillary obliteration		
Homocystinuria	Mental retardation		Dislocation of the lenses		Marfan-like abnormalities
MELAS	Generalized seizures, cognitive involvement, hearing loss, axonal multifocal neuropathy, migraine	Hypertrophic cardiomyopathy, Wolff-Parkinson-White syndrome	Eyelid ptosis, ophthalmoparesis, pigmentary retinopathy		Lactic acidosis, muscle weakness, exercise intolerance, diabetes mellitus, short stature
Familial hemiplegic migraine	Atypical long-lasting aura, ataxia				
Marfan syndrome		Aortic and cerebral artery dissection	Ectopia lentis		Pectus carinatum or excavatum, reduced upper-to-lower-segment ratio, scoliosis
Vascular Ehlers-Danlos syndrome	Intracranial aneurysms	Arterial dissection and rupture		Easy bruising, thin skin with visible veins	
Pseudoxanthoma elasticum			Angioid streaks	Increased elasticity, yellow-orange papular lesions	Hypertension
Sickle cell disease	Haemorrhagic strokes, seizures, spinal cord vascular events	Vaso-occlusive crises with pain			Hemolytic anemia, mild jaundice
Hereditary hemorrhagic telangiectasia	Arteriovenous malformations, embolic stroke, abscesses	Multiple arteriovenous malformation of bowel, lung, liver		Mucocutaneous telangiectases	Recurrent epistaxis

Neurologic events are described in up to 40% patients with pulmonary AVMs [69]. The commonest are TIAs or embolic stroke, as a consequence of right-to-left blood shunting associated to a pulmonary AVMs (cerebral paradoxical embolization) [70, 71]. TCD ultrasonography and transesophageal echocardiography, both with saline contrast, are useful in identifying patients with right-to-left shunts and may help, in turn, to suspect pulmonary AVMs [72]. Hemorrhagic stroke is also possible, due to rupture of a brain AVMs; the presence of multiple brain AVMs should raise the clinical suspicion of HHT [73]. Brain abscesses caused by septic emboli bypassing the filter of the pulmonary circulation and spinal AVMs are other findings in about 5% of HHT. Screening for pulmonary AVMs with TCD ultrasonography and preventive treatment should be recommended to patients with HHT [67].

CONCLUSION

Table 1 reports the clinical features commonly associated with stroke in the presented monogenic disorders. A complete clinical evaluation with the support of the brain imaging frequently allow the molecular diagnosis of a suspected monogenic stroke. Accurate diagnosis has therapeutic implications in some of these disorders, including Fabry, mitochondrial and sickle cell diseases.

Among the above mentioned monogenic causes of stroke, CADASIL, Fabry and mitochondrial diseases are the most common in the adult population. The minimum prevalence of CADASIL in general population is thought to be 1 in 25-50.000, with a mutation prevalence estimated at 4.14 in 100.000 and an individual risk of 1 in 13.500 to develop CADASIL in the general population [74]. Fabry disease is pan-ethnic and the reported annual incidence of 1 in 100.000 may underestimate its true prevalence [25]. It may explain approximately 1% of all strokes in the young [75, 76].

A simple and available method to screen for Fabry disease in large populations has been developed using fluorometric or tandem mass spectrometry of α -GalA activity in dried blood spots on filter paper [77, 78]. For mitochondrial disorders, the scenario is much more complex; about 10 in 100.000 people have clinically manifest mtDNA disease, making this one of the commonest inherited neuromuscular disorders; the m.3243A > G mutation has a minimum point prevalence of 1 in 6.135, being the most common pathogenic mtDNA mutation [79, 80]. The reported epidemiological data are likely to be underestimates, due to missed diagnoses of these rare disorders, so further studies are strongly needed in order to better define the impact of monogenic strokes in general population.

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Chapter 24

FABRY DISEASE: AN INTRODUCTION FOR NEUROLOGISTS

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ABSTRACT

Fabry disease (OMIM 301500) is an X linked lysosomal storage disorder due to deficiency of alpha galactosidase A (AGAL–A). Diverse mutations in the AGAL–A gene cause a multi-system disorder, often presenting in childhood but complicated by organ damage (principally renal failure, cardiomyopathy and cerebrovascular disease) and limitation of life expectancy in adulthood. Missense mutations in the gene are associated with residual enzyme activity and an attenuated form of the condition which typically presents in adulthood.

Neurological manifestations include small fibre neuropathy, abdominal pain, disturbances of eye and ear function, reduced sweating, depression, cognitive impairment and stroke or transient ischaemic attack.

Management of patients and families with Fabry disease requires a multidisciplinary team administering specific and supportive therapies. Treatment with enzyme replacement therapy has improved the outlook for patients and families with Fabry disease but there remains a need for improved specific treatment approaches.

INTRODUCTION

Fabry disease (OMIM 301500) is an X linked lysosomal storage disorder due to deficiency of alpha galactosidase A (AGAL–A) [1]. The condition was described simultaneously and independently by Johannes Fabry [10] and William Anderson [11], who both observed the characteristic skin rash as an important feature of the disease. Null mutations in the AGAL–A gene result in absent enzyme activity in males and boys typically

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present with features of classical Fabry disease. However, milder oligosymptomatic forms, presenting in adulthood and often affecting a single organ system, are increasingly recognised. These attenuated forms are associated with missense mutations and residual enzyme activity; adult males and females typically present to a range of adult physicians (e.g., nephrologists, neurologists and cardiologists) with signs of organ damage. The diagnosis is often delayed [40]. Neurological complications in childhood include acroparasthesia, hearing loss, tinnitus, cornea verticillata and abdominal pain [15]. Adults can present with stroke or transient ischemic attack at a young age (less than 55 years [27]). Fabry disease is also associated with a high prevalence of depression and older patients may develop cognitive impairment [38]. Two preparations of enzyme replacement therapy (ERT), agalsidase alpha (Replagal R, Shire HGT and agalsidase beta, Fabrazyme R, Genzyme/Sanofi) are licensed in Europe, Canada, Asia and South America while only agalsidase beta is approved by the FDA in the US [43]. Adjunctive care coordinated through designated expert centres and delivered by local physicians is recommended.

EPIDEMIOLOGY

Fabry disease (alpha galactosidase A, GLA, deficiency) in its classic form is generally considered the second most prevalent lysosomal storage disorder, after Gaucher disease [1, 2] with an estimated incidence ranging between 1:40,000 to 1:170,000 persons. All ethnic groups are affected. However newborn screening has revealed a higher incidence (about 1 in 3100 male births, [3]) of mutations in AGAL-A that are likely to be neutral but may result in a later onset and less severe phenotype. The incidence of Fabry disease among nearly 35 000 neonates screened in Austria is 1:3859 [4]. It may be estimated that the incidence of Fabry disease requiring treatment in Europe and North America is of the order 1 in 30-40,000.

ETIOLOGY AND PATHOGENESIS

Fabry disease is caused by the partial or complete deficiency of a lysosomal enzyme, α -galactosidase A. As a result of this enzyme deficiency, neutral sphingolipids with terminal α -galactosyl residues [predominantly globotriaosylceramide (Gb3)] accumulate in the lysosomes of different tissues and fluids (epithelial cells of glomeruli and tubules of the kidneys; cardiac myocytes; ganglion cells of the autonomic system; cornea; endothelial, perithelial, and smooth muscle cells of blood vessels; and histiocytic and reticular cells of connective tissue). Elevated levels of globotriaosylsphingosine (lyso-Gb3), which is the deacylated derivative of Gb3, have been found to correlate with disease severity in males and females [5]; and urinary levels of Gb3 have also been considered to be bio markers of the condition [6]. Lyso Gb3 has also been shown to stimulate the proliferation of vascular smooth muscle cells; and to be a mediator in signalling processes pathological significance in pain channels [7] and in kidney podocytes [8]. Lyso Gb3 has been shown to inhibit the activity of AGAL-A [5] and it has been suggested that elevated levels in females could be of pathogenic significance. Although the condition is X linked, heterozygous females are frequently affected and may be as severely affected as hemizygous males. Clinical symptoms typically

occur a decade or so later in females than in males and organ damage is usually less severe. The Lyon hypothesis is that one X chromosome is inactivated on a random basis in female tissues, while the other provides the genetic information. Skewed X chromosome inactivation, whereby the mutant X chromosome is expressed more frequently than the wild type, has been proposed recently as a major prognostic factor in heterozygous females [9].

The gene GAL for α -galactosidase A is located at the Xq22.1 region. More than 600 mutations have been identified in the AGAL-A gene [1], including missense, nonsense mutations and single amino acid deletions and insertions. Classic Fabry disease is typically associated with null mutations and absent enzyme activity in males. Non-classic Fabry disease is associated with mutations which encode enzyme of reduced activity or stability. Most of these mutations are 'private,' having been identified only in individual families. Some (e.g., the p.N215S mutation, which seems to have a high prevalence in the UK) have been associated with single organ (e.g., heart or kidney) or late onset variants and patients have normal levels of urinary Gb3, despite having clinical evidence of Fabry disease.

CLINICAL MANIFESTATIONS (TABLE 1)

Classic Fabry disease presents in boys from age 2-3 years with symptoms of a peripheral neuropathy - pain in the hands and feet (acroparasthesia) or cramping abdominal pain with diarrhea or constipation.

Late onset/single organ Fabry disease is much commoner. It presents in adulthood, the skin is rarely involved, there is predominant involvement of a single organ (kidney, heart or CNS) only, and the condition progresses more slowly than classic Fabry disease. Both forms of the condition can affect females; and X chromosome inactivation studies may help to predict the prognosis in females.

Skin and General Manifestations

These were highlighted in the original descriptions of the disease [10, 11].

Angiokeratoma (AK), are the cutaneous hallmark of Fabry disease [12] present in 70% of males and 39% of females. They may be widespread or grouped. In males lesions are typically within the 'bathing trunk' area (genitals, buttocks, lower abdomen, umbilicus, groins, inner thighs and sacrum). AK are also seen on the proximal limbs, particularly their medial aspects, the elbows and knees, the palms and soles, and over the distal phalanges of the digits and on the lips. In females AK are usually sparsely distributed and may occasionally occur in a dermatomal distribution. The commonest sites are the trunk and proximal limbs. Female genital lesions are relatively infrequent.

In both sexes, the presence of cutaneous vascular lesions (CVL), namely telangiectases and/or angiokeratoma has been associated with higher disease severity scores and a higher prevalence of major organ involvement [13]. These findings demonstrate the importance of dermatological assessment and its possible predictive value in terms of systemic morbidity.

Facial features: A "pseudo-acromegalic" facial appearance has been described in some families with Fabry disease and this appears to be commoner in patients with widespread angiokeratomas and classical disease at the more severe end of the spectrum [14].

Table 1. Clinical Manifestations

• Skin – Angiokeratoma – Telangiectasia – Raynaud’s phenomenon – Lymphodema – Hypohidrosis – Hyperhidrosis • Renal – Chronic renal failure • Cardiovascular – Hypertension – Coronary vascular disease – Left ventricle hypertrophy – Valve disease – Arrhythmias – Elevated high-density lipoprotein • Neurologic/psychiatric – Acroparesthesias – Cerebrovascular disease – Dementia – Depression – Increased suicide ideation • Ocular – Cornea verticillata – Conjunctival vessel aneurysms – Increased tortuosity of retinal vessels – Fabry cataract • Gastrointestinal – Diarrhea – Nausea – Vomiting – Postprandial flank pain – Malabsorption • Others – Hearing loss and tinnitus – Hypothyroidism – Obstructive airway disease – Osteopenia and osteoporosis – Anemia
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Lower limb oedema and lymphedema, particularly affecting the limbs, is frequently observed in Fabry patients. Lymphoedema was cited in the original description of FD [11] and Registry data from the Fabry Outcome Survey (FOS) on more than 700 patients confirms that reversible peripheral oedema of the lower limbs was present in 25% of Fabry males and

17% of females; lymphoedema was present in 16% of males and 7% of females. The mean age of onset was 37 years for males (range 13-70) and a decade or so later for females [12].

Abnormalities of sweating and temperature sensation: Reduced sweating is a classical feature of Fabry disease and thought largely to be a consequence of autonomic neuropathy, though substrate accumulation within sweat glands may play a role. Hypohidrosis was reported by 53% of males and 28% of females, with earlier onset in males (23 vs 26 years). Anhidrosis was described by 25% of males, but only 4% females [15]. Heat intolerance is a commonly associated and disabling symptom, resulting in reduced exercise tolerance, nausea, dyspnoea, light-headedness and headache, or complete collapse with loss of consciousness. Hyperhidrosis may also occur, more commonly in females than males: 11.9% of 369 females versus 6.4% of 345 males [16]. This is higher than the estimated prevalence of 1-2.8% of the general population in the US [34]. In a majority of Fabry patients it affects palms and soles and is not generalized. "Cold intolerance" and the development of pain in the extremities in cold environments is a frequent complaint amongst Fabry patients. More recently, Raynaud's phenomenon, has been documented in 8% of 710 females and 11% of 644 males [15]. This reversal of the sex ratio and high prevalence in males suggest a possible causal link to the underlying Fabry disease. This abnormal vasoreactivity of digital vessels could be related to autonomic dysfunction. Abnormalities of nitric oxide synthetase and increased oxidative stress in vascular endothelium and smooth muscle might also be important triggers [17] for Raynaud's and these observations may be relevant to the cerebrovascular circulation (see below).

Systemic Disease Manifestations

Fabry disease is a progressive, multi-system disorder which typically results in a global reduction in quality of life of affected individuals. Symptoms in childhood [18] typically relate to lethargy, tiredness, pain, cutaneous abnormalities, changes to sensory organs and often gastrointestinal disturbances. In early adulthood patients may suffer extension of any of the above symptoms and often develop lymphodema, proteinuria and the first signs of renal, cardiac or central nervous system/cerebrovascular disease. In late adulthood (age >30 years) symptoms include worsening of the above and more severe organ dysfunction (cardiac disease, renal disease and cerebrovascular disease).

Neurologic Findings

Acroparasthesiaes occur in 80-90% of affected individuals and typically occur in the first decade. Patients describe the sensation as pain, feeling like pins and needles in hands and feet often radiating proximally. Triggers include increased body temperature, exercise or stress. Pain typically declines over time and this may be due to damage to nerve fibres as a result of inflammation and substrate accumulation.

Sensory Organ Abnormalities

The commonest ocular finding is cornea verticillata (opacities in the cornea characterised by one or more lines radiating from the near the centre of the cornea) which occurs in over 90% of males and 70% of females. Other changes include increased tortuosity of retinal vessels, optic atrophy, cataracts and lenticular changes. The extent of ocular abnormalities correlates with the overall extent of the disease [19]. A recent study has also demonstrated genotype-phenotype correlations within the ocular manifestations, such that individuals with

nonsense mutations have more severe manifestations than those with missense mutations [20]. Tinnitus and high frequency sensory neural hearing loss are also common manifestations occurring in over 50% of subjects. Sudden deafness and dizziness due to vestibular pathology can also occur.

Gastrointestinal Changes

Cramping abdominal pain, nausea, diarrhoea and occasionally constipation are frequent and are often the presenting symptom [21]. The pathogenesis is probably related to neurologic abnormalities.

Organ Damage

Renal manifestations are seen in over 90% of males [22]. Micro-albuminuria and hyperfiltration are early features, proteinuria is typically seen in the third and fourth decades and progressive decline in renal filtering capacity occurs. Females frequently show proteinuria although progression to end stage renal failure is less common. A renal variant of Fabry disease has been described in patients with decreased, but not absent alpha-galactosidase A activity; these patients lack other characteristic manifestations [1].

Cardiac manifestations are a constant feature and increasingly recognised as the major cause of death in both male and female patients [23, 24]. Substrate deposition can be demonstrated throughout the myocardium, valves and conduction system and is often accompanied by an inflammatory cell infiltrate. A common presentation is with left ventricular hypertrophy but mitral valve prolapse, arrhythmia and coronary artery disease can all be present. A cardiac variant [1] has been described in patients with reduced but not absent alpha-galactosidase A activity and presents later in life (often above 40 years) with predominant cardiac symptomatology.

Stroke

Cerebrovascular manifestations include ischaemic or haemorrhagic strokes occurring early in life, transient ischaemic attack and strokes affecting the posterior circulation. Stroke is often reported as the presenting feature of Fabry disease and Fabry disease should be considered in the differential diagnosis of young patients with cryptogenic stroke. One European study [25] has suggested that up to 5% of males and 2.5% of females aged under 55 years with cryptogenic stroke may have Fabry disease; however, this has not been confirmed.

Stroke is frequently a devastating complication of Fabry disease and the cost effectiveness of ERT would be substantially improved if a clear impact of ERT on frequency and severity of stroke were demonstrable. The incidence of stroke in Fabry disease is much higher than in the general population, both in males and females, and strokes tend to occur at a younger age. Amongst diagnosed Fabry patients, the incidence of stroke and TIA is 7-11% of males and 4-6% of females respectively [26, 27]. Stroke is often the first manifestation of Fabry disease [7]. Typically, stroke occurs in advanced Fabry disease among patients who also have cardiac and renal disease, may be hypertensive, and frequently have other risk factors for thrombosis (e.g., Factor V Leiden [28]). Both haemorrhage and thrombosis can occur and there seems to be an increased incidence of strokes in the posterior circulation. The mechanism of stroke in Fabry disease is likely multifactorial and incompletely understood. Occlusive vasculopathy is certainly an important contributor to the pathogenesis of stroke in Fabry, as in all situations. Advanced atheroma has been demonstrated in a Fabry patient who

died of cardiovascular disease despite prolonged ERT [29]. The vascular tree is often markedly abnormal in Fabry disease and dolichoectasia is often observed. The posterior circulation is more often involved in Fabry patients than among stroke patients generally. The diameter of the basilar artery has been proposed as a marker of the degree of vascular abnormality in Fabry patients. Inflammation may play an important contributory role. Alterations in adhesion molecule expression leading to disturbed metabolism of nitric oxide synthase (NOS), oxidative stress [30] and increased peripheral blood myeloperoxidase have been reported [31].

The data on cerebral blood flow in Fabry disease are contradictory. Autoregulation of blood flow is known to be disturbed in Fabry disease. Global regional cerebral glucose utilization (rCMRGlu) is decreased and it would be expected that this would promote increased cerebral blood flow. Hilz et al. [32] used transcranial Doppler sonography to assess cerebral blood flow velocity (CBFV) and cerebral autoregulation in 22 Fabry patients (24 controls). Both parameters were reduced in Fabry patients compared to controls. In contrast, Moore et al. [33] used similar techniques and concluded that CBFV was actually increased in 26 subjects with Fabry disease and that ERT with agalsidase alfa reduced it toward normal levels. Uceyler et al. [34] prospectively studied 68 patients with Fabry disease by extracranial and transcranial Doppler sonography and concluded flow measurements were normal in Fabry patients and ERT had no impact on them. MRI scans in Fabry patients often show increased numbers of non-specific 'white matter lesions (WML) which are an indicator of vasculopathic damage. Fellgiebel et al. [35] examined serial MRI scans among Fabry patients being treated within the Fabrazyme phase IV study and reported that the WML burden was more likely to remain stable among the 25 ERT – treated patients than among 16 patients in the placebo group. Theoretically, one may imagine that if ERT has a positive effect on renal and cardiac disease, it may at least delay if not reduce the risk of stroke. However no convincing data have been produced to indicate an impact of ERT on stroke in Fabry disease. Adjuncts to ERT including good control of blood pressure, anti – arrhythmic therapy, aspirin or anticoagulants as appropriate are very important. By improving the plasma lipid profile, statins may be expected to reduce the risk of stroke in Fabry patients. Statins also have a range of beneficial effects on the different isoforms of NOS, as well as anti -inflammatory and anti-thrombotic effects; and they have been recommended for Fabry patients [36].

Other Manifestations

Global manifestations of Fabry disease include lethargy, tiredness, failure to thrive in children and anaemia. Depression is frequent and often under diagnosed, affecting up to 50% of Fabry males and females [37]. Sexual activity is often affected by the presence of angiokeratomas in the genital region and can lead to decreased self esteem and libido. Autonomic dysfunction is under recognised and can lead to hyperhidrosis, abnormal tear and saliva formation, abnormal cardiac reactivity, gastrointestinal dysmobility, altered pain and temperature perception and peripheral oedema. Endocrine abnormalities are uncommon but osteopenia and hypothyroidism are well described. Pulmonary abnormalities are under recognised; Fabry disease can lead to an obstructive airways pattern in some patients but asthma is the presenting feature in other patients. In contrast to other lysosomal storage disorders cognitive impairment is not generally seen, however, older patients (above 50 years) are increasingly seen with memory loss, global intellectual deterioration, and personality change [38].

DIAGNOSIS/DIFFERENTIAL DIAGNOSIS

The definitive diagnosis of Fabry disease is usually delayed, with a mean time between the onset of symptoms and diagnosis of 15 years. The diagnosis must be confirmed by the demonstration of deficient α -galactosidase A activity in plasma, serum, or leukocytes and the identification of the pathogenic mutation. Female patients have variable, and, occasionally, even normal levels of enzyme activity and DNA confirmation of the diagnosis is essential. Identification of a DNA change is necessary, but not sufficient as genetic variants of unidentified significance (GVUS) are frequently detected. Expression studies of the mutant can be helpful in demonstrating pathological significance [39].

Biopsy of tissues, such as skin and kidney, demonstrates the presence of lipid deposition and multilamellated myelin bodies in electron micrographs (see Figure). As Gb3 deposition starts in utero, prenatal diagnosis can be performed from chorionic villi or culture of amniotic cells, where low α -galactosidase A activity can be demonstrated [1].

The pain associated with acroparaesthesia is often misdiagnosed as rheumatoid arthritis, rheumatic fever, erythromelalgia, Raynaud disease, or simply as “growing pains” [40]. Such delay may allow development of irreversible organ damage.



Figure 1. Skin appearances in Fabry disease with extensive angiokeratoma.

PROGNOSIS AND CLINICAL COURSE

Earlier studies suggested that males with Fabry disease typically die in the 4th or 5th decade, and females live perhaps 15 years longer. More recently, the life expectancy for males has been estimated as 58.2 years (compared to 74.7 in the general US population) and 75.4 years for females (compared to 80 years) [41]. There is also evidence that, whereas renal failure was previously the commonest cause of death, cardiac disease and cerebrovascular disease are increasingly common [23]. Some of these changes may be due to the impact of ERT.

TREATMENT (TABLE 2)

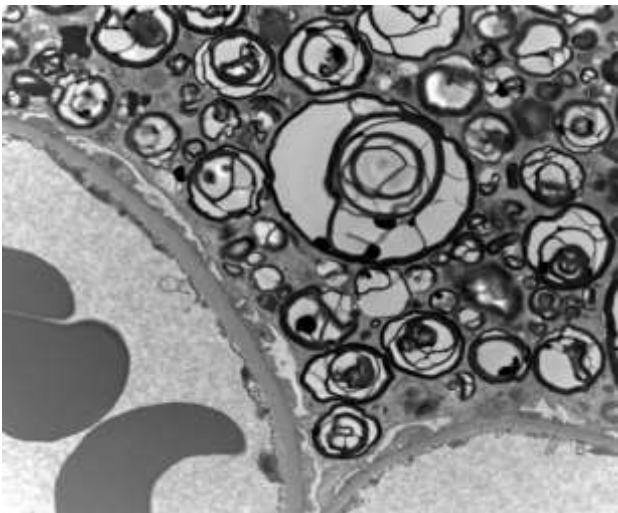
Fabry disease is a multi-system disorder and patients should be assessed within a multi-disciplinary setting. The advent of ERT has meant that patients and families are increasingly assessed in a specialist centre, where they will have access to a physician or paediatrician, dermatologist, and possibly cardiologist/nephrologist/neurologist [42]. Access to audiologists, ophthalmologists, gastroenterologists and psychiatrists/counselors, as well as to genetic counselors and nurses is desirable and available at larger centres. General aspects of supportive therapy may be as important as specific therapy.

Table 2. Treatment of Fabry Disease

SYMPTOMATIC
<i>Angiokeratomas</i> Liquid nitrogen, Electrocoagulation, surgical excision, Laser (pulsed-dye 585-nm, neodymium YAG 1064 nm, combined pulsed-dye and Nd:YAG) and IPL
<i>Lymphedema</i> Manual lymphatic drainage massage and compression
<i>Hyperhidrosis</i> Aluminium chloride hexahydrate, electrophoresis, Botulinum toxin, glycopyrrolate sodium
<i>Raynaud's phenomenon</i> Avoid smoking, cold and vasoconstrictor therapies, Losartan, diltiazem, fluoxetine, sildenafil
<i>Pain</i> Avoid triggers, Diphenylhydantoin, Carbamazepine, Gabapentin
<i>Stroke</i> Anti-platelet, Anticoagulant
<i>Hearing</i> Hearing aid devices, Avoid noise trauma

Table 2. (Continued)

<i>Lungs</i> Avoid smoking, Bronchodilators
<i>Gastrointestinal</i> Pancrelipase, Metoclopramide
<i>Cardiovascular</i> Antihypertensive drugs, Antiarrhythmia drugs, Artificial pacemakers, Implantable defibrillators, Coronary bypass
<i>Chronic renal failure</i> Angiotensin converting enzyme inhibitors Hemodialysis, Allograft transplant
SPECIFIC Enzyme replacement α -Galactosidase B (Fabrazyme), α -Galactosidase A (Replagal)



Zebra bodies

Figure 2. Electron micrograph in Fabry disease showing characteristic ‘zebra’ bodies.

Therapies Directed at Other Organ Systems [42, 43]

Pain is the most disturbing and early symptom, and can sometimes be partially managed with diphenylhydantoin, carbamazepine, or gabapentin. Patients should be encouraged to identify and try to avoid their personal precipitating factors.

For the primary or secondary prevention of stroke and other vascular pathologies, such as retinal artery occlusion, anti-platelet and anticoagulant therapy might be needed.

Metoclopramide and pancrelipase are used to reduce gastrointestinal symptoms. Hypertension must be controlled, as it significantly affects three of the most affected organs, the kidney, brain, and heart. Angiotensin receptor blockade should be undertaken at the first sign of proteinuria and is an important adjunct to ERT in slowing the decline in renal function. Fabry patients are good candidates for kidney transplant in the event of ESRF and the role of ERT in this situation is to preserve the function of other organs. Ancillary care from a cardiac perspective includes consideration of antiarrhythmics, artificial pacemakers, and surgery including septal ablation or even cardiac transplant.

Specific Therapeutics: Enzyme Replacement Therapy

This has been recently reviewed [43]. Two formulations of enzyme replacement therapy have been developed, agalsidase beta (Genzyme, Massachusetts US) and agalsidase alpha (Replagal, Shire HGT). Only agalsidase beta is licensed in the US whereas both formulations are available in most other parts of the world. Agalsidase beta is administered at a dose of 1.0 mg/kg biweekly and is manufactured using a recombinant technology in a Chinese hamster ovary (CHO) cell line. Agalsidase alpha is given at a dose of 0.2 mg/kg biweekly and is manufactured using a gene activation methodology in a human fibroblast cell line. The efficacy of both ERT formulations has been demonstrated in randomized controlled trials [44, 45] and both improve biochemical (e.g., levels of Gb3 in plasma, urine and tissue biopsy) and clinical parameters. The main clinical parameters chosen for study are renal function, pain, cardiac size and function and quality of life. Longer term trials have been reported for both preparations. Long term effectiveness has been demonstrated in Registry studies of agalsidase alpha [46] and agalsidase beta [47]. Both preparations are considered safe and well tolerated. The main side effects are infusion related (fever, temperature) and both preparations can induce antibody formation. Although an impact of antibodies on clinical effectiveness has not been conclusively demonstrated, a recent study suggests they may be important [48]. A large multi-centre study from Canada suggests the two ERT formulations are of equivalent effectiveness [49]. The optimum time for commencement of treatment has not been established but a recent consensus statement [50] has emphasized the importance of early intervention before the onset of irreversible organ damage, in both males and females. ERT is recommended for all symptomatic males and at the first sign of organ dysfunction in females. Patients receiving ERT should be regularly monitored with serial measurements of pain, quality of life, renal and cardiac function. ERT has been shown to improve various aspects of neurologic function including pain, hearing [51], peripheral nerve and sweat function [52] and GI symptoms [53]. The only direct beneficial effects of ERT on CNS abnormalities to have been demonstrated are improvements in blood flow disturbance [33] and reduction in WML burden [35]. Infused enzyme cannot cross the blood brain barrier.

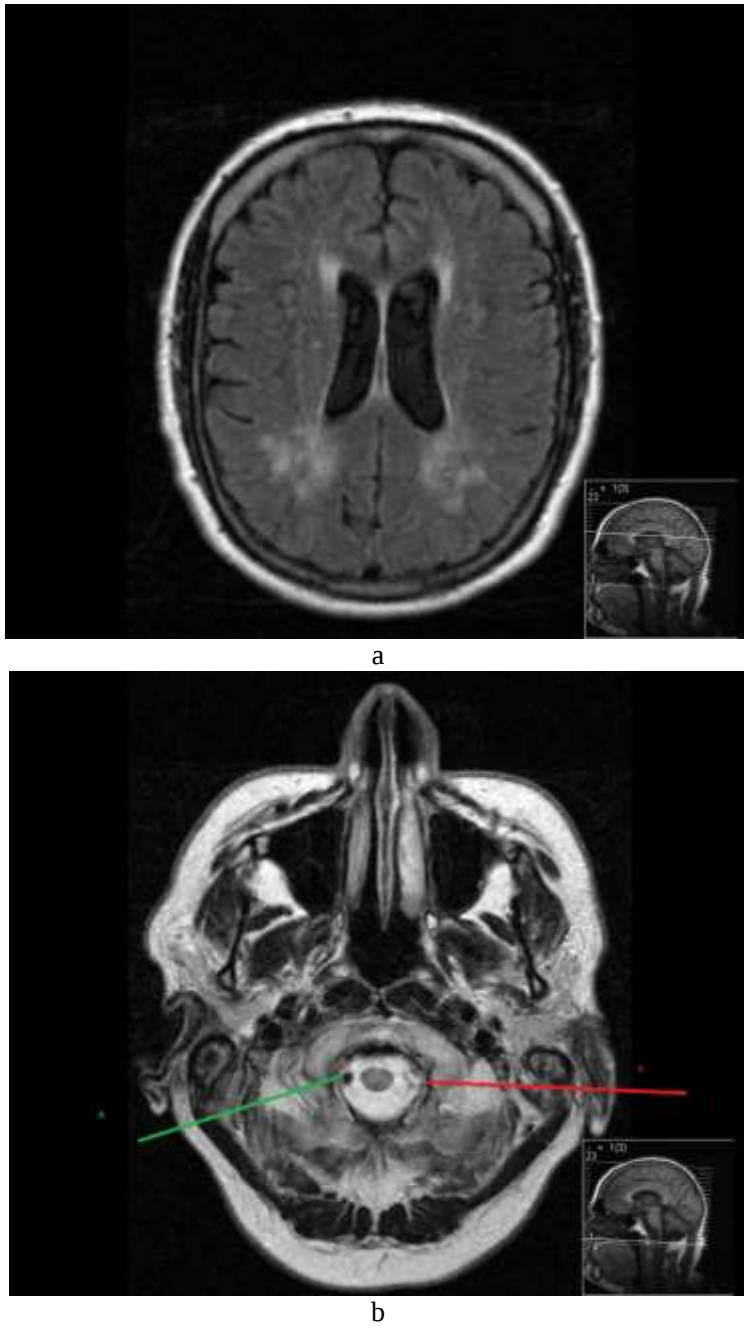


Figure 3. (a) MRI scan to show white matter lesions on FLAIR sequences. (b) There is marked asymmetry of the vertebral arteries and the distal left intracranial segment has a reduced lumen with reduced flow (see arrows); this is due to structural tortuosity of the artery, which is a frequent finding in Fabry disease.

New Treatments

Chaperone-based enzyme enhancement therapy consists of small molecules that rescue misfolded/mistrafficked enzymes from the lysosomes and transport them to the endoplasmic reticulum. Chaperone-based therapy can be administered orally but is useful only for patients with residual enzyme activity [54]. Substrate deprivation (inhibition of an early step in the synthesis of glycosphingolipids) and the infusion of galactose are other therapeutic options that are still in the research state. Gene therapy and hematopoietic cell transplantation are being actively developed [55].

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Chapter 25

PATHOGENESIS OF HEMORRHAGIC STROKE

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ABSTRACT

Intracerebral hemorrhage is the least treatable and often fatal form of stroke. Conventionally, ICH is classified as ‘traumatic’ or ‘spontaneous.’ The spontaneous group, which represents the 80% of cases, is further subdivided into ‘primary,’ when an underlying cause is not identified, or ‘secondary.’ Spontaneous ICH occurs more frequently in males, elderly and Africans or Asians and hypertensive subject. The most important causes of spontaneous ICH are deep perforating vasculopathy, which occurs in nearly 50% of cases, and cerebral amyloid angiopathy, detected in 5-20% of ICH patients. However, given the complexity of the pathophysiology of ICH, including inflammatory pathways, apoptosis, mitochondrial dysfunction acting according to the disease stages, a clear pathogenic mechanism has not still defined. Since ICH is now recognized to be a manifestation of the small vessel disease, it is believed that ICH derived from the interaction between the common cerebrovascular risk factors as well as environmental and genetic factors and vulnerable and fragile vessels.

Keywords: cerebral hemorrhage, risk factors, pathogenesis, genetics, neuroimaging markers, causes, deep vasculopathy, small vessel disease, cerebral amyloid angiopathy, neoplasm, intracranial vascular malformation, cavernous malformation, Moyamoya

INTRODUCTION

Intracerebral hemorrhage (ICH) is defined as a focal collection of blood within the brain parenchyma. It is the second most common form of stroke, accounting for 10-30% of first-ever strokes. A recent meta-analysis including 8145 patients estimated an overall incidence of

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ICH of 24.6 per 100,000 person-years (95% CI 19.7–30.7) and the incidence did not decrease over time [1]. Hospital admission for ICH increased by 18% in the last 10 years, probably due to the increased number of elderly and increasing use of anticoagulants, antiplatelets and thrombolytics [2-3]. ICH is one of the most devastating stroke forms leading to be a major cause of morbidity and mortality worldwide [4]. In fact, despite a significant improvement in the management of ischemic stroke, ICH remains associated with a very high case fatality rate (from 13% to 61%) in the first month and only 38% of patients have been estimated to survive over the first year.

Conventionally, ICH is classified as ‘traumatic’ or ‘spontaneous.’ The spontaneous group, which represents the 80% of cases, is further subdivided into ‘primary,’ when an underlying cause is not identified, or ‘secondary’ (due to identified causes including bleeds into tumors, cavernomas, arterio-venous malformations, CNS infection, cerebral venous sinus thrombosis, bleeding disorders, etc.) [5].

ICH may be classified also as deep (45%), lobar (30-40%) and infratentorial (15%). The site of hemorrhage and its extension to other compartments such as subarachnoid, subdural or intraventricular may be useful for identifying the cause.

RISK FACTORS

Spontaneous ICH occurs more frequently in males, elderly and Africans or Asians [3-8]. Hypertension is the major risk factor for spontaneous intracranial hemorrhage (about 65% of cases) and the risk is higher in young subjects and raises with increasing blood pressure values. In these cases ICH is typically observed in deep brain structures [6-7]. Up to half of patients with hypertension suffering from ICH are unaware of their hypertension and/or they do not assume adequate therapy. Alcohol intake is another risk factor for ICH acting in dose-dependent manner, with higher risk among those subjects with a high daily alcohol intake.

Diabetes mellitus and current smoking are considered weak risk factors [6]. Data regarding lipid levels are controversial since serum triglyceride levels (≤ 0.4 mmol/L) have been associated with an increased risk of ICH [9].

Interestingly, ICH related to oral anticoagulants as well to sympathomimetic drug abuse [10] is increasing. In subjects under warfarin the ICH risk-per-year is 0.3%-1% per patient-year, while a noteworthy increase of ICH risk is detected when the international normalized ratio is > 3.5 [11].

NEUROIMAGING MARKERS

It is now generally accepted that the so-called ‘primary ICH’ represents a feature of small vessel disease [12-13]. Neuroimaging, and particularly MRI, is important not only for distinguishing hemorrhage from ischemic stroke in the acute phase but also to identify some important markers of small vessel disease (SVD) such as leukoaraiosis and microbleeds [13].

In fact, despite major hemorrhage and in some way lacunar strokes can be quite easily observed by conventional CT scan appearing like low density lesions, MRI plays a crucial role in detection of microbleeds and leukoaraiosis [14-15]. White matter lesions (WMLs) are

best seen on T2 weighted MRI scans (including FLAIR sequences) as more or less confluent hyperintense areas bilaterally sited in the hemispheric white matter. T2-weighted and FLAIR MRI have been widely used to quantify also WML load [15]. WMLs are typical both of hypertensive arteriopathy and CAA, with similar lesion distribution [16-17].

Lacunar infarcts, which are frequently associated with WMLs, are identified as small, frequently round or ovoid shapes, CSF density filled cavities with a diameter of 3 to 10-15 mm [18]. Upper limit of 1.5-2 cm differentiate them from striatocapsular and embolic subcortical strokes [14], whereas lower limit of 0.3-0.5 cm is used to differentiate them from smaller perivascular spaces. However, enlarged perivascular spaces (EPVS) were found to be associated with WMLs and lacunar stroke suggesting they could be another feature of SVD [19]. EPVS can be seen acutely on diffusion weighted imaging or as hypointense foci surrounded by an hyperintense rim on MRI T1-weighted and FLAIR sequences typically located in the areas supplied by the lenticulostriate or pontine paramedian arterioles, such as basal ganglia, internal capsule, thalamus and pons but also diffusing in hemispheric white matter [20-21].

MRI technique such as T2*-weighted gradient recalled echo (GRE), sensitive in detection iron deposition, can detect small foci of old hemosiderin deposits, called cerebral microbleeds (CMBs), appearing as black or hypointense lesions. CMBs correspond to focal accumulation of hemosiderin containing macrophages in the perivascular spaces surrounded by inflammatory reaction with activated microglial cells, complement activation, apoptosis and sometimes gliosis [22]. CMBs are characterized by the so-called 'blooming' effects for which areas of GRE MRI appear greater than the actual hemosiderin deposits. Despite some authors attempted to classify microbleeds with a maximum diameter from 2 to 10 mm [23], the available literature support the idea of the limited utility of size definition. Recently the use of higher resolution techniques such as three-dimensional GRE MRI, which is able to detect more CMBs than conventional one, supported the idea that different reported prevalences depend from the utilized methodology [24-26]. In fact microbleeds are paramagnetic and induce a susceptibility effect on MRI scan, depending from length of TE as well as their diameter and blooming effect [24]. However, several criteria for CMBs detections have been suggested [15]. CMBs prevalence has been also associated with severity of leukoaraiosis and lacunar infarcts [26]. Moreover, CMBs are considered a reliable predictor of future lobar hemorrhage and cognitive decline [27].

The location of CMBs may be helpful in determining the etiology of SVD since deep CMBs reflect hypertensive arteriopathy whereas lobar CMBs are more frequently associated with CAA [16]. Molecular imaging using PET amyloid ligands suggest that lobar CMBs preferentially occur at site of A β concentration [28].

PATHOLOGICAL FINDINGS AND PATHOPHYSIOLOGY

Different pathophysiological phases, which are mainly resumed in primary and secondary injury, have been described in ICH.

Primary Injury

ICH, which results from rupture of small penetrating arteries damaged by hypertension (hyalinosis, lipohyalinosis) or from rupture of pial branches in the case of cerebral amyloid angiopathy (fibrinoid necrosis), is generally surrounded by edema, but also inflammation and necrosis [29]. By mechanical compression hematoma may induce a disruption of the neuron and glia architecture, which in turn provides a blood flow neurotransmitter release impairment and a mitochondrial dysfunction [30-32]. This early phase correlates with initial clinical symptoms and signs.

Concomitantly the hemostatic cascade initiates and clot and hemoglobin breakdown products activate microglia, which in turn releases neurotransmitters inducing breakdown of the blood-brain barrier, vasogenic oedema, and apoptosis in neurons and glia. This phase correlates with hematoma expansion, which occurs in almost 73% of patients within 3-12 hours, and perihematoma oedema growth, which occurs within the first 24 hours from ICH onset. In this phase mitochondrial and metabolic dysfunction, as well as hypoperfusion phenomena, have been observed followed by a variable reperfusion phase from 2 to 14 days after ICH [33-37].

Secondary Injury

Secondary injury after ICH may be caused by different mechanisms, including: 1) direct complications of the hematoma mass effect such as hydrocephalus, 2) increased intracranial pressure leading to decreased cerebral perfusion and tissue shifts [4, 38] and 3) release of clot and inflammatory components (i.e thrombin, hemoglobin and iron), already initiated by the primary injury [4]. These factors may exert toxic effects on endothelial cells, neurons, astrocytes and microglia, contributing also to disrupt the blood brain barrier [39].

CAUSES OF ICH

Spontaneous ICH

The most important causes of spontaneous ICH are deep perforating vasculopathy and cerebral amyloid angiopathy.

Deep Perforating Vasculopathy

Nearly 50% of ICH is due to deep perforating vasculopathy, which results probably from reactive hyperplasia and microscopic degenerative changes of vessel wall components leading to reduced vascular reactivity and enhanced vessel wall fragility [40-41]. Deep perforating vasculopathy usually occurs in lenticulostriate arteries originating from middle cerebral artery as well as in small thalamic arteries arising from the posterior communicating and posterior cerebral arteries, and perforating vessels arising from the basilar artery. Spontaneous ICHs, which are due to rupture of small deep arteries, occur most frequently in the deep portion of cerebral hemispheres and the most common location are putamen (35-50% of cases),

subcortical white matter (30%) and cerebellum (16%). Pontine hemorrhages account for 5-12% of cases [40, 42]. In the past years hypertension related degenerative changes were believed to be the most important pathogenic mechanism. More recently other mechanisms involving environmental and genetic factors have been invoked [43]. One hypothetic pathogenic mechanism is that multiple acute or chronic risk factors, mostly sustained hypertension, interact with vulnerable damaged small vessels, leading ultimately to rupture and thus to ICH. However, a recent population based study reported that ICH might result from short-term increases in blood pressure prior to the event (over weeks to months), suggesting that blood pressure control may effectively reduce the risk of ICH [44].

Cerebral Amyloid Angiopathy

Sporadic cerebral amyloid angiopathy (CAA) accounts for at least 5% to 20% of all spontaneous ICH. It is characterized by the progressive deposition of amyloid β ($A\beta$) within the wall of small/medium size arteries and lesser in capillaries and veins in the leptomeninges of cerebral cortex [45-46]. This process, that preferentially affects cortical-subcortical regions, and rarely cerebellum and deep or brainstem structures, induces a thickening, focal fragmentation and disruption of small vessels, that sometimes is associated with microaneurismal dilatation and luminal occlusion [46-47]. Other neuropathological features associated with CAA, such as cortical microinfarcts and white matter hyperintensities, probably reflect reduced perfusion phenomena by CAA-affected arteries [48]. These aspects result in a wide spectrum of small vessels disease manifestations varying from asymptomatic lacunar lesions and cerebral microbleeds to symptomatic stroke, leukoaraiosis and ICH [13, 46]. Several evidences from pathological studies support the hypothesis that CAA has an important role in causing ICH [49-50]. A recent meta-analysis of published histopathological studies confirmed the association between CAA and lobar ICH (OR: 2.21; 95% CI: 1.09–4.45) [51]. A pathological study comparing brains with CAA and with and without ICH found that ICH subjects had a more severe CAA picture with fibrinoid necrosis in comparison to non-ICH subjects [51]. Microaneurysms occurred only in the presence of severe, rather than moderate or mild, CAA [52].

CAA has been described more frequently in elderly, in Eastern population [53-54] and does not seem to be associated with common cerebrovascular risk factors whereas anticoagulants and antiaggregants have been found to increase the risk of CAA related ICH [55-56].

The Boston criteria were proposed to estimate the likelihood of CAA in ICH. These criteria were mostly based on the site and number of hemorrhagic lesions on neuroimaging [57]. However, although these criteria have been widely used, some limitations due to the gap between the original analyzed sample (only symptomatic ICH) and the wide range of clinical and neuroradiological CAA findings were reported [51].

Secondary ICH

Several conditions have been identified as possible cause of ICH, and should be always considered in the diagnostic work up. They include brain injuries, primary and secondary neoplasms, aneurysms, arterio-venous malformations, cavernomas, coagulopathies, dural sinus venous thrombosis, vasculitis, Moya-Moya disease, brain irradiation and medications such as anticoagulants, thrombolytics, antiplatelets but also sympathomimetics and illicit drugs (cocaine, ecstasy amphetamines) (Table 1)

Table 1. Secondary causes of ICH

Traumatic
• <i>Immediate</i>
• <i>Delayed</i>
Aneurysm
Arterio-venous malformations
Cavernomas
Neoplasm with hemorrhage
• <i>Primary (glioblastoma, haemangioblastoma)</i>
• <i>Secondary (melanoma, histiocytoma, Grawitz's, lung, etc)</i>
Coagulopathies
Leukemia, DIC
Thrombocytopenia and other platelet disorders
Hemophilia and other hereditary coagulopathies
Hepatic failure
Renal failure
Dural sinus venous thrombosis
Artero-venous dural fistulae
Vasculitis
Vasculopathies
Dissections
Moya-Moya
Surgical procedures and interventions
Neurosurgery
AVM surgery
Craniotomy
Subdural haematomas burr-hole evacuation
Spinal surgery
Neuroradiological interventions
Carotid endoarterectomy
Carotid angioplasty
Cardiac transplantation
Brain irradiation
Sonothrombolysis
Medications
• <i>Anticoagulants, Thrombolytics, Antiplatelets</i>
• <i>Sympathomimetics, including over-the-counter pills and nasal sprays (ephedrine, pseudoephedrine and phenylpropanolamine)</i>
Alcohol
Illicit drugs (Cocaine, Ecstasy, Amphetamine)

Cerebral Arteriovenous Malformations (AVM)

VM are abnormal connections between arteries and veins, leading to arteriovenous shunting with the presence of the so-called “nidus” (network of vessels). The prevalence of AVM is between 15 and 18 subjects per 100 000 adults [58]. The overall detection of incidental AVM rate is 1 per 100 000 adults per year [59]. The first-ever hemorrhage rate is 0.55 per 100 000 person-years [59]. The hemorrhagic risk is higher (4.5%-34%) in previously ruptured brain AVMs (with deep venous storage and deeply seated); the risk is lower in unruptured brain AVMs (0.9%-8%) [60-61]. The risk of subsequent hemorrhage is higher in

case of: 1) deep venous drainage, 2) brain AVM presentation with hemorrhage, 3) association with aneurysms, 4) deep location [61-62].

Cavernous Malformations (CMs)

Cavernous malformations (CMs) consist of endothelium lined dilated spaces with deficiency in both the smooth muscle layer and the tight junction and with occurrence of thrombosis and calcification. Prevalence of CMs is 0.4–0.6% [63-64]. CMs account for approximately 5%–10% of all cerebral vascular malformations, and roughly 20% of them are located in the brainstem [64-65]. Most CMs remain clinically quiescent. CMs located in neurologically eloquent areas are prone to become symptomatic often with seizures due to the microscopic accumulation of hemosiderin in susceptible cortex. CMs can be sporadic or hereditary. CMs can cause extra-lesional hemorrhage (beyond the confines of the cavernoma) or intra-lesional hemorrhage (within the cavernoma itself). In patients managed conservatively hemorrhage risk varies between 0.25% and 4.5% per person/year [64]. The overall risk of first hemorrhage in familial cases is 1.4% per person/year. The risk of recurrent hemorrhage after a first intracerebral hemorrhage has been evaluated from 3.8% to 33.9% per year [64]. In brainstem CMs an annual secondary hemorrhage rate of 5.1% has been found in patients not receiving treatment [64-65].

Moyamoya Disease (MMD)

Moyamoya disease (MMD) is a chronic occlusive cerebrovascular disease involving progressive stenosis of the terminal portion of the internal carotid arteries and/or the proximal portions of its main branches, associated with the development of fine collateral networks, especially adjacent to the site of occlusion in the deep areas of the brain. This last feature, detectable on dynamic angiography, was originally described in the Japanese literature as ‘moyamoya,’ which translates into English as “a puff of cigarette smoke” [66-67]. MMD is a rare disease more frequently occurring in Asian countries, particularly in Japan where an incidence up to 0.54 per 100000 has been reported. However, although MMD has been described in all races and ethnicities worldwide, limited data are available in Western countries, where the incidence rate is probably about ten times lower (about 0.086 per 100000) than in Asia [68-69], and it probably remains a misdiagnosed cause of ischemic or hemorrhagic stroke.

Cerebrovascular events are the main presenting symptoms and are related both to the stenosis and occlusion of the ICAs (transient ischemic attack, ischemic stroke) and to the rupture of fragile collateral vessels (hemorrhagic stroke). In the Japanese literature, opposed to what observed in US, the ischemic type has been shown to predominate in childhood, while the hemorrhagic type is more often observed in the adult population. In Western countries ischemic events seem predominate both in children and in adult patients [70-74]. In adult MMD intracerebral hematomas are more often associated with intracranial aneurysms, ACA occlusion, fetal-type PCA, MCA occlusion with collateral flow, whereas ACA occlusion was mostly associated with deep intracerebral hemorrhage or IVH [75-76]. Independent risk factors for hemorrhage are anterior choroidal artery and posterior communicating artery dilation [75], cerebral microbleeds mostly near the midline structure or in deep brain areas. The annual risk for stroke (hemorrhagic or ischemic) is 3.2% per year in asymptomatic Moyamoya patients [76]. In symptomatic Moyamoya patients, the 5-year risk of recurrent ipsilateral stroke is 65% if medically treated, and 17% in the group admitted to

surgery [76]. In the Japanese population, during a 5-year follow-up in hemorrhagic Moyamoya, rebleeding attacks were observed in 11.9% and in 31.6% of patients who were surgically and conservatively treated, respectively [77].

GENETICS OF ICH

Single-Gene Disorders

A number of monogenic disease may predispose to cerebral hemorrhage with two main mechanisms: 1) favoring the development of macroscopic vascular malformation such as mutations in the malcavernin and KRIT1 genes associated with CCM1 and CCM2 or in the ENG gene and other loci associated with Hereditary Hemorrhagic Telangiectasia (HHT) 2) causing cerebral hemorrhage in the context of a more complex picture of small vessels disease such as Hereditary Cerebral Amyloid Angiopathy and mutations in COL4A1 gene and, less often, in NOTCH3 gene, associated with CADASIL [78].

Cerebral Cavernous Malformation (CCM)

Cerebral cavernous malformations (CCMs) consist of abnormally enlarged capillary cavities in the absence of intervening brain parenchyma. Three genes have been recognized as responsible of heritable CCM: KRIT1 (CCM1;OMIM 604214-116860), CCM2/malcavernin (CCM2; OMIM 607929) and PDCD10 (CCM3;OMIM 609118). The pattern of inheritance is autosomal dominant. The most common clinical symptoms are seizures, cerebral hemorrhage, focal neurological deficits and headache with onset around thirty years [79] with an earlier occurrence of Haemorrhagic symptoms [79].

Hereditary Hemorrhagic Teleangiectasia (HHT)

The disease is characterized by the presence of multiple arteriovenous malformations (AVMs) that lack intervening capillaries and result in direct connections between arteries and veins. It is a genetically heterogeneous condition with an autosomal dominant pattern of inheritance and age-related penetrance. So far a number of different genes have been identified such as endoglin (ENG) gene (OMIM 187300-131195) on chromosome 9q34.11, activin A receptor type-II like 1 (ACVRL1) gene (OMIM 600376-601284) on chromosome 12q13.13 and SMAD4 gene (OMIM 175050-600993) on chromosome 18q21.2. The main clinical findings are epistaxis, gastrointestinal bleeding, telangiectasia, pulmonary and hepatic AVMs. Central nervous system involvement occurs in about 10% of cases [80].

Hereditary Cerebral Amyloid Angiopathy (H-CAA)

1) APP-Related CAA (OMIM 605714-104760)

The APP gene, located on chromosome 21q.21.3, encodes for amyloid precursor protein (APP; 104760). Mutations in the APP gene cause abnormal deposition of A-beta peptide.

Hereditary cerebral hemorrhage with amyloidosis–Dutch type (HCHWA-D) is an autosomal dominant form of CAA due to a point mutation at codon 693 of the A-beta precursor protein gene (glutamine for glutamic acid substitution at position 22 of A-beta.

Mutation carriers develop diffuse white matter changes, brain hemorrhages and ischaemic strokes. Dementia is common after 40 years and is associated with extensive amyloid-induced vasculopathy affecting cortical vessels independently of neurofibrillary tangles or plaques [81-82]. Typical neuropathological findings consist of diffuse amyloid fibril depositions in the wall of cerebral and leptomeningeal small and medium size vessels with limited neurofibrillary degeneration and in the absence of classical dense core A-beta plaques [83]. Severe pathological features of CCA with or without neurofibrillary pathology have been observed in the Italian (E693 K), Arctic (E693G), Iowa (D694 N) and Piedmont variants (L705 V) [84]. The Flemish mutation (A692G) results in a phenotype characterized by early onset AD or cerebral hemorrhage with neurofibrillary tangles and AD-type parenchymal plaques centered on blood vessels [84].

2) Cystatin-Related (CST3), Icelandic Type, CAA (OMIM 105150-604312)

HCHWA-I is an autosomal dominant form of CAA resulting from mutations (A to T point mutation causing a glutamine for leucine substitution) in the cystatin C gene on chromosome 20p11.2. The clinical picture is characterized by early onset ICH and dementia. ICHs are often multifocal and are more likely to occur in previously healthy and normotensive subjects [85-86]. Cystatin is produced by neurons and other CNS cells and is an inhibitor of extracellular cysteine protease [86]. The amyloid protein isolated from leptomeninges of affected patients starts at position 11 of normal cystatin C and has an amino acid substitution, Leu to Gln, at position 68 (L68Q) leading to a change in some physical properties of the protein resulting in accelerated cerebrovascular amyloid deposition [87]. Pathological studies reveal widespread deposition of amyloid resulting from degradation products of mutated cystatin C in leptomeninges and brain, but also in peripheral tissues such as skin, lymphoid organs, salivary glands and testes [87-88].

3) TTR-Related CAA (OMIM 105210)

TTR related amyloidosis are a genetically and phenotypically heterogeneous group of autosomal dominant clinical entities due to mutations on the TTR gene on chromosome 18. Systemic involvement includes polyneuropathy, carpal tunnel syndrome, autonomic insufficiency, cardiomyopathy, and gastrointestinal features, less frequently accompanied by vitreous opacities and renal insufficiency [88-89].

A distinct form of TTR-related amyloidosis with predominant CNS involvement is leptomeningeal amyloidosis. The main clinical features are dementia, seizures, strokes and visual impairment secondary to vitreous amyloid deposition. Hagiwara et al. (2009) reported a patient with progressive polyradiculoneuropathy, ataxia and hearing loss who developed lethal multiple cerebral hemorrhages [90]. Diffuse amyloidosis involving leptomeninges and subarachnoid vessels associated with patchy fibrosis and obliteration of the subarachnoid space were evident on pathologic examination. Associated findings were severe and diffuse neuronal loss and generalized subpial gliosis and occasional superficial brain infarcts [91].

COL4A1 Syndrome (OMIM 605595-120130)

Type IV collagen is a major component of basement membranes consisting of six homologous, but genetically distinct, α chains that are selectively expressed in different membranes and at different stages of embryonic development [92]. COL4A1 gene, consisting of 52 exons, is located on the telomeric region of chromosome 13q (13q34) by Solomon et al.

(1985) and Emanuel et al. (1986) [93-94]. Mutations in the collagen IV genes are associated with reduced stability and defects of the basement membrane. The resulting phenotype is consistent with a systemic small vessel disease with recurrent brain involvement. Mutations in the collagen IV alpha chain 1 (COL4A1) gene are known to be associated with a highly variable phenotype consisting of familial porencephaly, migraine with and without aura, infantile hemiparesis, seizures and perinatal intraventricular hemorrhages [95-97]. Clinical features of small vessel disease of the brain are quite common findings. Stroke occurs in 17.3% of subjects with a mean age at onset of 36.1 (range 14-40 yrs.). The most part is hemorrhagic (67%) whereas all the ischaemic strokes are lacunar (33%).

COL4A1 gene has been recently associated with sporadic ICH [97]. In a cohort of 96 patients with sporadic ICH (48 deep and 48 lobar ICH) COL4A1 gene has been sequenced and 2 rare nonsynonymous variants were found. It has been proposed that altered COL4A1/2 secretion may contribute to sporadic cerebrovascular disease and ICH [97].

Candidate Gene Variants Associated with Intracerebral Hemorrhage

As for other complex diseases, a number of polymorphisms, mostly implicated in vascular wall integrity (i.e APOE, ACE, neprilysin, endoglin, TGF β 1), endothelial function (i.e ACE), vessel wall reactivity or coagulation (i.e APOE, CD-14, Factor VII, XIII, VKORC1) have been investigated as potential risk factors for sporadic cerebral hemorrhage, using the candidate-gene approach. However, due to some methodological problems the results of these studies are controversial and were not robustly replicated [78].

Vessel Wall Integrity

APOE (locus 19q13.2): Apolipoprotein E is a glycoprotein that plays an important role in the lipid transport and metabolism, in the cellular membrane stability and reparation and it is significantly expressed in the brain. Moreover, ApoE plays a critical role in several other processes, such as neuronal damage reparation, maintenance of synaptic plasticity, mitochondrial resistance to oxidative stress and the use of glucose in neuronal and glial cells.

The well-known relationship between APOE and Alzheimer's disease (AD), which is strictly associated to cerebral amyloid angiopathy CAA (80% of subject affected by AD show some evidence of CAA), led to a growing interest in studying the APOE frequency in patients with CAA-ICH.

Apolipoprotein E gene, mapped on chromosome 19, encodes for a glycoprotein, ApoE, with three common isoform E2, E3, E4, codified by ϵ 2, ϵ 3, ϵ 4 alleles, whose frequency in Caucasian population are, respectively, 7,3%, 78,3% e 14,3% [98]. These three different allelic forms (ϵ 2, ϵ 3, ϵ 4) differ for a cysteine/arginine polymorphism in 112 and 158 positions and can generate 6 different genotype; ϵ 3/ ϵ 3 genotype is the most represented and is present in about 50-75% of the population.

Many studies have been carried out aimed at investigating the role of APOE polymorphism in Haemorrhagic stroke, with contrasting results.

Greenberg et al, in 1996 [99] firstly demonstrated the association between ApoE ϵ 4 and pathologically proven or suspected cases of cerebral amyloid angiopathy, as well as with an earlier onset of lobar hemorrhage. They also demonstrated that ApoE ϵ 4 is neither necessary nor sufficient for the occurrence of CAA-related hemorrhage. In fact the vast majority of

subjects carrying this allele in general population do not show clinical CAA. Otherwise other studies [100-101] demonstrated the association between ApoE $\epsilon 2$ and not $\epsilon 4$ allele and ICH and lobar hemorrhage.

Both these ApoE alleles would seem to increase the risk of CAA, and are also associated with the occurring of the first Haemorrhagic event at a young age and a higher recurrence rate. According to Greenberg (1998) [99] and Nicoll (2001) [101] ApoE $\epsilon 4$ acts in CAA by promoting amyloid deposition. ApoE $\epsilon 2$, instead, is not associated with amyloid deposition, but rather to degenerative changes, such as fibrinoid necrosis, which leads to breakage of those vessels in which the amyloid is deposited. Considering these mechanisms, these two alleles appear to have a complementary role in CAA progression. Sudlow et al. 2006 [102] reported that ApoE $\epsilon 2$ was associated with the occurrence of ICH (OR1.32; 95% CI 1.01-3.87) and the association was particularly strong for lobar hemorrhage (OR 2.37; 95% CI 1.45-3.87). Recently, in a three-phase study (one discovery and two replication studies), Biffi et al. in 2011, [103] demonstrated that individuals carrying ApoE $\epsilon 2$ and ApoE $\epsilon 4$ alleles had lobar hemorrhage, but only ApoE $\epsilon 2$ was associated with lobar ICH volume (mean increase 5.3 mL; 95% CI 4.7-5.9) and poor outcome (OR 1.68; 95% CI 1.10-2; $p=0.009$) and mortality (OR 1.60; 95% CI 1.13-2.25; $p=0.008$). The generally accepted explanation is that ApoE- $\epsilon 4$ promotes vascular amyloid deposition, while ApoE- $\epsilon 2$ promotes progression to severe CAA with associated vasculopathic changes, which cause vessel rupture and ICH [104]. ApoE $\epsilon 2$ allele was also found to be correlated with leukoencephalopathy, but neither $\epsilon 2$ nor $\epsilon 4$ allele seems to be related to the presence of microbleeds on MRI [105]. A study on 41 elderly patients with warfarin-associated cerebral hemorrhage showed a higher frequency of $\epsilon 2$ carriers, especially in patients with lobar ICH [106].

ACE (Locus 17q23) and α -ADDUCIN (Locus 4p16.3)

The angiotensin-converting enzyme (ACE) is a dipeptidyl carboxypeptidase that catalyzes the formation of angiotensin II from angiotensin I and of bradykinin [107]. Both these substances are mediators of vascular tone and are involved in smooth muscle cell proliferation, in vascular remodeling and in atherosclerosis. Angiotensin II is a potent vasoconstrictor involved in vascular hypertrophy, thrombosis and vessel wall damage [108]. Human ACE gene contains 26 exons and is localized on the chromosome 17. Studies on human ACE gene identified a polymorphism that consists on the presence (insertion, I) or absence (deletion, D) of a 250 base-pairs fragment in intron 16, whose presence or absence influences the enzyme activity, with the DD genotype correlated to a greater activity, the II genotype to a lower activity and the ID genotype to an intermediate one.

So far there are no convincing data on the role of ACE gene I / D polymorphisms as a risk factor for spontaneous intracerebral hemorrhage. Two studies on Asiatic, Japanese and Taiwan population yielded negative results whereas a polish study reported a positive association between ICH and ACE gene [109-111].

In an Indian study conducted in 2011, the ACE DD genotype, alone or in association with a single nucleotide polymorphism, providing the substitution of tryptophan in place of a glycine of Alfa-adducin (ADDI), was associated with spontaneous ICH, especially not lobar [112]. The presence of DD genotype showed a risk of ICH 7.4 times higher than wild genotype (OR 7.04, $P 0.001$). The presence of D allele was significantly associated with ICH compared to I allele.

Coagulation System

Factor V Leiden (Locus 1q23) and Prothrombin 20210A (Locus 11p11-q12)

A missense mutation in codon 506 of the factor V gene (called FV Leiden) was associated with resistance to activated Protein C (APC). This molecular variant creates a prothrombotic state, which is believed to be a risk factor for venous thrombosis. Subjects with factor V Leiden molecular variant had decreased risk for spontaneous intracranial hemorrhage (odds ratio, 0.19; 95% confidence interval, 0.03-0.95)(118). Prothrombin 20210A/G is another frequent prothrombotic single nucleotide polymorphism (SNP), which was associated with reduced risk of ICH in comparison to controls (1.5% vs 3%, respectively) [113].

Factor VII (Locus 14q34)

Factor VII plays an important role in coagulation system and its deficiency is associated with bleeding disorders. The congenital deficit of this protein has been observed to predispose to spontaneous (including intracranial) and post-surgical bleeding. It has been suggested that a polymorphism conditioning the insertion/deletion of 10 base pairs in position 323, that is associated with lower levels of factor VII, could predispose to cerebral hemorrhage [113]. However, the available data are not sufficient to draw significant conclusions.

Factor XIII (Locus 6p25.1)

Coagulation factor XIII is a transglutaminase, which plays an important role in the clot stabilization through the crosslinking of fibrin chains, in matrix remodelling, cellular adhesion and migration and tissue repair. Congenital factor XIII deficiency is a hereditary bleeding disorder caused by reduced levels and activity of factor XIII, which is characterized by bleeding diathesis with a clinical manifestation often including intracranial hemorrhage. Studies on sporadic cerebral hemorrhage revealed that a point mutation G → T in exon 2 codon 34 of the gene encoding the A subunit of FXIII, which involves the replacement of a Leucine with a Valine (FXIII Val34Leu), seems to increase the risk non-traumatic cerebral hemorrhage [114-115].

However, the role of FXIII Val34Leu is controversial since other studies and the meta-analysis of Peck et al. [116-117] did not confirm the association between this molecular variant and ICH.

Serpin 3 (Locus 14q32.1)

SERPIN 3 gene (formerly known as α 1-antichymotrypsin) encodes a protease inhibitor protein, which could prevent the degradation of extracellular matrix in the vessel wall. In a study on the Spanish population the association between the A/T polymorphism in SERPIN 3 gene and cerebral hemorrhage has been reported in normotensive patients [118-119]. Conversely a Polish study did not find any association between the SERPIN 3 A/T polymorphism and the risk of cerebral hemorrhage [120]. Although ethnic differences in genetic background and in susceptibility to cerebral hemorrhage may explain the heterogeneous results, available studies are not enough convincing to get any significant conclusion.

MTHFR (Locus 1p36.3)

The methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in the transformation of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which acts as a methyl donor for methylation of homocysteine to methionine, through the intervention of vitamin B12. The homocysteine metabolism is partially determined by common SNPs in the MTHFR gene, such as C677T and A1298C, which seem to have a role as risk factors for stroke. Some studies have reported that high plasma levels of homocysteine are associated not only with ischemic stroke, but also with cerebral hemorrhage [121-123], by effects on coagulation and by rupture of microaneurysms. In any case, data relating the role of homocysteine and MTHFR genotype in Haemorrhagic stroke are currently few and have low significance. Peck et al, in 2008 [117] found a weak association between MTHFR 677TT genotype and hemorrhagic stroke within overall OR of 1.11 (95% CI 0.89-1.39). A recent prospective study showed that both hyperhomocysteinaemia and MTHFR 677 T allele are independent risk factors for hemorrhagic stroke in the Swedish population [124]. Other explored factors involved in the coagulation system such as Serpin 1 (locus 14q32.1), Endoglin (locus 9q34.1), Glycoproteins Ia (5q11.2), IIIa (17q21.32) and Ib α (17p13.2) Perilipin (locus 15q26) were not associated with the risk of ICH [78, 117].

Inflammatory Markers

Since inflammatory response plays an important role following ICH, and release of inflammatory cytokines increases blood-brain barrier permeability, cerebral oedema and secondary neuronal injury, a number of candidate gene involved in inflammatory process was studied.

TNF (6p21.3 Locus)

The Tumor Necrosis Factor α is one of the main proinflammatory cytokine and plays a central role in initiation and regulation of the inflammatory response. Increased values of serum TNF were associated with an increased rate of mortality after ICH and recurrent stroke. A study conducted on Taiwan population investigated the possible role of four SNPs in the promoter region of this gene (T-1031C, C-863A, C-857T, and G-308A) in predisposing to deep brain hemorrhage or influencing the outcome at 30 days [115]. The study demonstrated a weak association between these variants and the risk and the size of ICH.

IL-4 (Locus 5q31.1)

Interleukin-4 (IL-4) is a member of the T helper 2 (Th2) cytokines and its role is to reduce the production of pro-inflammatory cytokines and destructive enzymes by monocytes [126]. IL-4 polymorphisms (rs2243250, rs2070874) were analyzed in Korean patients with intracerebral hemorrhage (ICH) [127]. In this study, IL-4 gene polymorphisms [rs2243250 (promoter 524T/C) and rs2070874 (5-UTR, 33T/C)] were associated with ICH by lowering IL-4 levels and its neuroprotective effect [128].

IL-6 (Locus 7p15.3)

IL-6 plays a key role in promoting acute inflammatory response and regulating the production of acute phase proteins such as C-reactive protein. In addition, it contributes to the inflammatory response by activating endothelial cells and stimulating the synthesis of

fibrinogen. It has been demonstrated that the 572G → C polymorphism is significantly associated with the risk of cerebral hemorrhage [130]. Another polymorphism within the same gene (174G → C) was found to be associated with cerebral hemorrhage secondary to an arteriovenous malformation [130]. It was also demonstrated that high IL-6 levels are a predictive factor of increased hematoma volume in the acute phase [131]. These observations suggest that IL-6 is important both in onset and in the evolution of cerebral hemorrhage. The local release of IL-6 could contribute to vascular wall instability by stimulating the release and the activation of matrix metal-proteinases (MMPs).

ALOX5AP (Locus 13q12.3)

Among eicosanoid biosynthesis genes (ALOX12, ALOX5, ALOX5AP, PTGES, PTGIS, PTGS1 and PTGS2) ALOX5AP was found to be associated with stroke and to contribute to ICH susceptibility in Korean population [132]. In this study, the G allele frequency of rs17222919 in the ICH group was about 3.8-fold (86.7%) than controls. These results suggest that the G allele may be a risk factor for the development of ICH.

Complement Cascade

Common genetic variants in the key components of complement pathway have been associated with greater inflammation and cerebral injury after ICH. A prospective cohort study was implemented to investigate the relationship between SNPs in complement component 3 C3 (rs2230199, R102G, C/G), complement component 5 C5 (rs17611, I802V, A/G) and Complement Factor H (rs1061170, Y402H, T/C) genes and mortality after ICH [133]. Complement Factor H (CFH) inhibits pathologic activation of the inflammatory cascade, and thus cellular damage. Functional genetic variants in CFH can result in aberrant CFH activity, leading to abnormal activation of the complement cascade, greater degrees of inflammation in the perihematoma zone, and worse brain injury and outcome. Genetic variation in CFH, in particular the Y402H polymorphism, was significantly associated with both discharge ($p = 0.01$) and 6-month mortality ($p = 0.02$) after spontaneous intracerebral hemorrhage, whereas this association was not observed for C3 ($p = 0.545$ and $p = 0.830$) or C5 ($p = 0.983$ and $p = 0.536$) SNP.

PDGF-D (Locus 11 q22.3)

Platelet-derived growth factor D (PDGF-D) plays an important role in angiogenesis, vessel remodelling, inflammation and repair in response to injury. The -858AA genotype associated with higher risk of first-ever ICH in non-hypertensive cases, as well as reduced incidence of recurrent ICH [134]. This effect may be explicated by the angiogenic and wound healing effects of PDGF-D.

TP53 (Locus 17 p13.1)

TP53 encodes for a tumor suppressor protein, which mediates apoptosis (p53). The presence of apoptotic neurons in the area surrounding hematoma in hemorrhagic strokes may be a cause of reduced recovery. Several SNPs have been identified within the TP53 gene, both in the coding and in the non-coding region. One of the most studied is a single nucleotide polymorphism in codon 72 (exon 4) with an arginine-proline substitution (Arg72Pro) in a domain involved in the preapoptotic function of p53. The Arg72 variant of

p53 enhances the properties of the proapoptotic protein and inhibition of oncogenesis compared to variant Pro72. It was recently demonstrated an association between the genotype Arg/Arg and a poor prognosis after ischemic or hemorrhagic stroke. This genotype was also correlated with an increased volume of the cavity resulting from cerebral hemorrhage. The *in vitro* Arg72-p53, but not the Pro-p53, interacts directly with mitochondrial Bcl-xL and activates the apoptotic intrinsic pathway, increasing the vulnerability to apoptosis induced by ischemia. These results suggest that the TP53 genotype Arg/Arg influences the neuronal vulnerability to apoptosis and may be a genetic marker of poor outcome following an ischemic or hemorrhagic stroke [135].

Tubulin β 1 (Locus 20b q13.32)

Platelets play very important functions in hemostasis and atherothrombosis. Genetic alterations in platelet receptor proteins have been studied as potential risk factors for thrombotic events or bleeding. The β 1-tubulin is expressed in platelets and in megakaryocytes and, together with α -tubulin, forms heterodimers that are assembled in microtubules polymers. It has been demonstrated that the β 1-tubulin is essential for platelets formation although the exact mechanism by which the β 1-tubulin participates in platelet aggregation has not yet been elucidated. A TUBB1 Q43P polymorphism has been observed to increase the risk of cerebral hemorrhage. The platelet hyporeactivity in carriers of this allele may be particularly relevant in patients taking antiplatelet drugs such as aspirin. It has been observed that the presence in the same subject of TUBB1 Q43P and FVII 323 Del/Ins polymorphisms increases the risk of developing cerebral hemorrhage of about 20 times, suggesting that the combination of low factor VII plasma levels with reduced platelet activation could have dramatic effects. Also the association with the FXIII L34 allele increases the risk of developing ICH, through the association of a defect of platelet aggregation and a decreased stability of the clot [136].

CR1 (Locus 1q32.2)

A genome-wide association study conducted in order to investigate the genetic risk factors for sporadic AD demonstrates that a variant within the CR1 gene (single nucleotide polymorphism rs6656401) increases the risk for Alzheimer disease (AD). Given the pathological overlap between AD and CAA-related ICH, Biffi et al. [137] recently performed a case control study in order to determine whether this polymorphism also influences the risk of developing clinically symptomatic CAA-related ICH, as well as histopathological severity of CAA. They found out an association of the A allele of rs6656401 at the CR1 locus with the risk of CAA-ICH (OR 1.61, 95% CI 1.19–2.17, $p = 8.0 \times 10^{-4}$), the risk of recurrent CAA-ICH (HR 1.35, 95% CI 1.04–1.76, $p=0.024$), and the severity of CAA pathology at autopsy (OR 1.34, 95% CI 1.05–1.71, $p=0.009$). According to this study, CR1 would seem to increase the risk of both AD and CAA acting independently on vascular and parenchymal amyloid deposition.

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Chapter 26

ANIMAL MODELS OF STROKE: PRESENT AND FUTURE

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ABSTRACT

Animal models have greatly contributed to our understanding of the risk factors and the pathophysiology of stroke, as well as the development of therapeutic strategies for its treatment. Further development and investigation of experimental models, however, are needed to elucidate the pathogenesis of stroke and to enhance and expand novel therapeutic targets. In this chapter, we provide an overview of the characteristics of commonly-used animal models of stroke, which may provide insights into a framework for developing effective therapies for stroke in humans.

Keywords: animal model; stroke; ischemia; neuro-inflammation.

INTRODUCTION

Over the last four decades, a variety of animal stroke models have been developed, with the aim of identifying the pathophysiologic mechanisms that underlie cerebral ischemia and developing new agents for stroke therapy. Several ischemic stroke models have been developed in a variety of species; these include mechanical occlusion of the middle cerebral artery (MCA), thromboembolic models, and photothrombotic models.

This chapter focuses on models of global and focal cerebral ischemia by means of vessel occlusions.

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VESSEL OCCLUSIONS

Because brain function depends on cerebral blood flow (CBF) for continuous supply of energy metabolism, brain ischemia can be modeled by occlusion of a cerebral arterial vessel.

The residual CBF caused by vessel occlusion can be graded according to two major variables: time and space. Time refers to the duration of the occlusion. Space refers to the extent of the lesion, according to the site of the occlusion. There are, therefore, models of temporal or permanent occlusion, and models of focal or global ischemia models. While global ischemia is consistently associated with a temporal occlusion of bilateral large vessels of the neck, focal lesions distribute along a continuum, which ranges from very short to permanent occlusions affecting small or large brain volumes. The extent of the ischemic lesion depends on whether the occlusion is proximal or distal. Occlusion of the vessel is obtained by mechanical or biochemical means. Direct mechanical occlusion include ligation or clipping, or insertion of a silicon-coated nylon threads into the internal carotid artery that is subsequently advanced to the target vessel. In order to mimic thromboembolism, macrospheres or autologous blood clots are injected directly into the internal carotid artery. Local injection of purified thrombin or of vasoconstrictor substances (such as endothelin-1 in the rat) may cause reproducible focal lesions. Pros and cons of these approaches have been described in several reviews [1] and the choice of a model reflects the balance between reproducibility, similarity with pathophysiology of stroke, easiness and brevity of the approach, extent of surgery and anesthesia (Table).

Global Ischemia

Global, permanent brain ischemia causes irreversible whole brain damage and death. Reversible, temporary global ischemia, such as it occurs in humans during cardiac arrest and resuscitation, is a transient loss of blood flow to the entire brain. If the global ischemia is short enough the brain tissue survives the insult, but parts of the tissue or nervous cells may eventually die, hours or days following the insult. The phenomenon is modeled by the classic 2-vessel occlusion in the gerbil. Bilateral occlusion of the carotids in the gerbil causes marked, diffuse reduction of CBF. A long lasting (approximately more than 15 minutes) occlusion causes death. Shorter occlusions may appear asymptomatic or result in minor behavioral disorders. Typically, following 5-minute occlusion the animals show no evident motor or behaviour deficit, to eventually develop in the days following the occlusion a focal lesion in the CA1 section of the hippocampus. Remarkably, CBF returns back to normal a few minutes after the release of the occlusion. Thus the pathogenic process that would end in cell death occurs under conditions of normal CBF. Two phenomena, therefore, characterize the 2-vessel 5-minute occlusion in the gerbil: 1) an interval of time during which there is no structural change before the damage becomes apparent 2-4 days following recirculation (maturation phenomenon); 2) a focal distribution of the damage in spite of the whole brain ischemia (selective vulnerability). The finding indicates that intrinsic tissue factors contribute heavily to the damage. Severe hypoxia or hypoglycemia may also produce brain injury in specific brain areas, consistently with the notion that local variables, other than blood flow, are relevant in determining the vulnerability to the energy deprivation.

Table 1. Advantages and limitations of rodent models of focal and global cerebral ischemia

	<i>Advantages</i>	<i>Limitations</i>	<i>Experimental procedure</i>
<i>Focal ischemia</i>			
Craniotomy	High long-term survival rates; Visual confirmation of successful MCAo	Invasiveness; High degree of surgical skill	Direct surgical MCAo requiring craniectomy and section of the dura mater to expose MCA
Endothelin-1 (ET-1)	Non invasive	Variability of magnitude and duration of ischemia; Astrocytosis and axonal sprouting; Not suitable for thrombolysis studies	Direct application of vasoconstrictor ET-1 to MCA
Embolic stroke	Similarity with pathogenesis of human stroke; Appropriate for studies of thrombolytic agents	Variability of lesion size; Low reproducibility; Spontaneous recanalization	Injection of autologous blood or artificial clots into ICA
Intraluminal suture	Non invasive; Suitable for transient and permanent ischemia	Risk of post stroke hyperthermia or subarachnoid hemorrhage	Filament advanced via the ICA to occlude MCA
Clip/mechanical device occlusion model	Control of occlusion site; Reproducible infarcts; Suitable for transient or permanent ischemia; Low mortality	Invasive	Clipping of MCA
Photothrombosis	Non invasive; Reproducible infarcts	Infarct size usually small; Not suitable for ischemia in clinically relevant arterial territory (MCA)	Injection of photoactive dye; Laser irradiation of cerebral cortex; Microvascular coagulation in irradiated area
<i>Global ischemia</i>			
2 vessel occlusion in the gerbil	Easy to perform; Non invasive	Model of cardiac arrest	Bilateral occlusion of CCA
4 vessel occlusion in the rat	Easy to perform	High mortality rates; Uncertain complete occlusion of vertebral arteries	Electrocauterization of vertebral arteries and after 24 hours bilateral occlusion of the CCA

MCA: Middle Cerebral Artery; MCAo: MCA occlusion; ICA: Internal Carotid Artery, CCA: Common Carotid Artery.

The report of the phenomenon [2, 3] has boosted research in the field, as it became evident that ischemic damage is not just a sudden event causing necrosis. It can be a process triggered, but not sustained, by the initial ischemic insult. In general, the length of the maturation process is related to the intensity of the insult: the stronger the insult the shorter the delay in the development of the structural damage. The ischemic damage of the CA1 hippocampus associated with 5 min bilateral carotid occlusion in the gerbil is prevented by 2+2 min occlusion (*per se* not deleterious) carried out at 48 h interval before the 5 min occlusion [4]. The 2+2 min occlusion serves as a subliminal ischemic insult, which induces tolerance to ischemic injury. It is the result of the activation of endogenous adaptive mechanisms, which follow the exposure (preconditioning) to a stressful, but non-damaging, stimulus. Ischemic tolerance can be acquired by several stimuli including short episodes of transient focal and global cerebral ischemia, remote organ ischemia, hypoxia, hypothermia, hyperthermia, exposure to inhalation anesthetics, cortical spreading depression, brief episodes of seizures or by exposure to low-dose bacterial lipopolysaccharide prior to cerebral ischemia [5–7].

Bilateral occlusion of the common carotid arteries in the young-adult rats is without any apparent acute, or sub-acute pathogenic effect. The condition is instead thought to model the chronic hypoperfusion frequently observed in aging and neurocognitive progressive disorders [8]. Thus, the so-called 2-vessel occlusion model in the rat has been employed to examine the role of cerebral hypoperfusion in neurodegenerative processes [9]. A similar model was developed in the mouse by positioning microcoils in both carotid arteries [10]. Occlusion of the arteries causes decrease of CBF to 35–45% of control in cortical areas and to about 60% in the hippocampus and subcortical areas. A partial recovery of the flow occurs in the days following the occlusion with an almost complete recovery in 2–3 months. After 6 months the flow has completely recovered in the study by Choy et al. [11]. The cerebral glucose utilization seems to follow the reduction in CBF, with an initial delay and a more rapid recovery [12, 13]. Thus, the 2-vessel occlusion animals show an initial acute CBF reduction followed by progressive recovery. The progressive restitution to normal flow, therefore, encompasses a phase of chronic oligoemia, associated with reduction of glucose metabolism. Such a phase lasts for 2–3 months following vessel ligation. Because of such long-lasting recovery, the pattern of blood flow changes is inconsistent with the constant or progressive cerebral hypoperfusion observed in aging and dementia. In order to model the chronic blood flow derangement, gradual occlusion of the carotids with a device that constricts over time has been used [14].

In spite of such a limit, the chronic oligoemia is thought to model the chronic hypoperfusion observed in humans, and to cause similar structural and functional brain changes. The 2-vessel occlusion rats show behavioral changes, which share similarities with the cognitive disorders in humans. The animals show impaired spatial and non-spatial memory and fear conditioning [9]. The behavioral alterations are measurable a few weeks following occlusion and seem to progress with time in spite of the recovery of the blood flow. The progression is with the hypothesis that most of the changes occur during the chronic phase of the hypoperfusion, consistently with the hypothesis of a neurodegenerative-like mechanism sustained by the long-lasting hypoperfusion.

Focal Ischemia

Focal, permanent, or long lasting, occlusion of an intracerebral vessel causes necrosis, which coexists with brain areas of altered function, containing damaged, yet viable, cells. In a classical experimental setting [15, 16] clipping of middle cerebral artery in the baboon, during anesthesia, causes drop in regional CBF, subsequent moderate elevation of extracellular K⁺, extracellular acidosis and decline in evoked potential amplitude. By varying systemic blood pressure, and hence regional CBF, the authors were able to establish the interrelations occurring among regional CBF, evoked potential, extracellular K⁺ and pH. Reduction of CBF to approximately 15 ml/100gm per minute (approximately 35% of control) in the baboon leads to complete failure of the somatosensory evoked response. Lower rates of CBF were associated with massive release of intracellular K⁺ (as detected by intra-cerebral electrodes), a condition likely reflecting cell death. Thus, different low levels of regional CBF were associated with different biochemical and functional consequences. Essentially, two CBF thresholds were identified in ischemia: the lower threshold for release of K⁺ (necrosis), and the higher threshold for electrical failure (dysfunctional, viable cells).

The findings introduced the concept of ischemic penumbra, defined as a peri-necrotic area in which, and for a certain time interval, the neurons remain structurally intact but functionally inactive. A major finding was that by increasing regional CBF the authors could reverse the electrical failure. Thus, a key issue in the definition of penumbra is the reversibility of the functional failure. Such a key feature is difficult to evaluate in animal models. Since the original definition, the concept of penumbra has evolved to include potentially measurable characteristics, including electrophysiological, biochemical or molecular alterations [17]. For instance, the expression of Hsp70 is being considered a potential marker of the penumbra, in animal models. The expression of Hsp70 in neurons outside areas of infarction is presumed to protect these cells from further protein denaturation, and most cells that express both Hsp70 mRNA and Hsp70 protein appear to survive. Histochemistry for Hsp70 is therefore being considered a reliable tool for identifying in laboratory animals the penumbra area. Other approaches have been used to identify the penumbra, such as protein synthesis, blood flow or metabolic changes defined by a variety of imaging methods [18]. Evaluating differences between regions of abnormal tissue perfusion and diffusion regions on MRI [19] is probably the most common approach in clinical setting. This approach, however, presents flaws and does not directly address the penumbra definition. Most of these approaches are results of conceptualizations aimed to validate an operational definition of penumbra, such to be able to visualize the phenomenon and determine the size and time, in the contest of clinical practice, or at least in the setting of trials.

Traditionally the penumbra is depicted as an area circumscribing the necrotic core. Authors have recently stressed the limit of such a model by emphasizing the heterogeneity of the penumbra itself. In animal models of ischemia there are small islands of the infarcted brain (mini-cores) surrounded by neurons and glial cells that express Hsp70 (mini-penumbra) [17]. The 'most-stressed' cells at the margins of these islands are sometimes neurons and sometimes microglia. We do not know whether such heterogeneity occurs also in humans following stroke, but it is a substantial hypothesis that in vivo neuroimaging approaches underestimate such heterogeneity, and fail in visualizing small islands of recoverable brain tissue within the most affected brain areas.

A number of local and systemic variables affect the outcome of brain cells within the penumbra area. The most relevant variables, as mentioned, are the entity of the local residual CBF and the time interval of the blood flow reduction. There is, therefore, interaction of severity and duration of ischemia in the development of irreversible cell damage. In experimental animals, the penumbra occurs when local CBF is reduced to 20-40% of control (contralateral) values, and the area might be as large as one half of the entire early ischemic lesion [20]. Infarction, in humans, usually corresponds to local CBF values below 12 mL/100g/min and the irreversible damage is thought to occur in a few minutes if the flow is near zero or in 30-60 min if it is close to 12. Penumbra occurs in areas where local CBF decreases to 12-22 mL/100g/min [21].

This is, however, quite a gross approximation as other variables, in addition to intensity and duration, affect the susceptibility of the tissue to the ischemic damage. Such variables include temperature, systemic inflammation, aging or glucose serum levels.

Limits to the Models

Most of the animal models have been carried out in young, healthy animals. There are relatively few reports that emphasize the potential role of variables not directly linked to the obstacle to blood circulation. Considerable interest has been recently devoted to post-ischemic inflammation. Abnormal conditions or diseases, systemic or apparently isolated to other organs, may affect the brain inflammatory response. As a consequence a number of variables not directly associated with the brain ischemia might boost the maturation process or increase the brain tissue liability to the ischemic insult. It is important to appreciate that inflammation starts in the vessel triggered by hypoxia, shear stress and ROS production. Both innate and adaptive immunity is then involved and leads to brain tissue infiltration of inflammatory cells. These inflammatory cells damage the brain by generating cytotoxic mediators and promote programmed cell death. Inflammation embraces a number of different mechanisms, within a complex scenario. For instance, microglia can proliferate and migrate. Activated microglia can assume a primarily cytotoxic phenotype called M1, or an antiinflammatory, protective phenotype called M2. Likely microglia fluctuate in these activation phenotypes during the evolving ischemic process.

Chronic inflammation from atherosclerosis, autoimmune disease, and physiological stress may cause production of proinflammatory cytokines, which diffuse into the brain via the circumventricular organs and activate the resident resting microglia. Resident, immune-competent cells in the brain tissue are likely primed by systemic infection to promote the brain tissue damage following an ischemic insult. Peripheral cytokines, in contact with the endothelial cells that make up the BBB, cause release of various immune molecules into the parenchyma, including NO, prostaglandin E2, IL-1 and IL-6.

Aging itself might prime the microglia. A strong support to the influence of systemic variables on the development of focal ischemic lesions comes from experimental studies carried out by using transplant of mesenchymal stromal cells (MSCs). A large body of evidence indicates that MSCs may aid in reducing the long-term impact of stroke, via multiple mechanisms that include induction of angiogenesis, promotion of neurogenesis, prevention of apoptosis, thus driving plasticity and neurovascular remodeling [22]. However, recent data, in animal models, show that only a very small number of transplanted MSCs

localize to the area of the ischemic lesion. A key feature of MSCs seems to rely on the remote effect that these cells can have. The hypothesis of a remote effect has been strengthened by recent evidence that stem cells release extracellular vesicles. MSCs do in fact release large amounts of exosomes, endocytic small-membrane (30-100 nm) vesicles. These vesicles elicit biological activity similar to the stem cells themselves [23, 24]. Environmental challenges, such as activation or stress conditions, influence the composition, biogenesis, and secretion of exosomes, which likely represent a means by which distant organs or conditions may affect the brain tissue altering its susceptibility to insults.

Animal models currently used are limited in modeling systemic variables that might increase the susceptibility to ischemia. There are, however, a few relevant exceptions. Spontaneously Hypertensive/Stroke Prone Rats develop diffuse brain pathologic changes that mimic subcortical vascular disease [25, 26]. Transgenic mice expressing mutated NOTCH3 protein present dysfunction of the neurovascular units lower threshold for cortical spreading depression, NOTCH3 extracellular domain and osmiophilic deposits, and white-matter lesions resembling CADASIL.

All together, the data gathered in the last 2 or 3 decades, confirm that the residual blood flow associated with occlusion of a vessel is the main but not unique variable causing the brain damage. Furthermore the structural outcome is not the unique variable that affects the prognosis. Together with improvement in methodology for focal or global cerebral blood flow reduction, experimental set up should include modeling of local or systemic variables, which likely affect susceptibility to ischemia, maturation of the post-ischemic damage and restoration and repair.

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Chapter 27

THE ADDED VALUE OF NEUROSONOLOGY: A TAX-FREE GENIUS IN THE PROBE

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ABSTRACT

Neurosonology is essential for stroke neurologists, as it can play a key-role in the diagnostic and therapeutic work-up of patients. In this chapter, two challenging issue will be discussed: carotid stenosis (in particular, grading and plaque characterization) and hyperacute revascularization (in particular, intracranial occlusion identification and sonothrombolysis), underlining strengths and limitations of ultrasound in such relevant clinical settings.

Keywords: neurosonology, carotid stenosis, stroke, sonothrombolysis

INTRODUCTION

Neurosonology represents a very informative and relatively unexpensive diagnostic and therapeutic tool, which can be easily implemented at patient's bedside. Its excellent safety, in addition to great spatial and real-time resolution, allows frequently repeatable examinations and monitoring. Operator-dependency, which is related to any type of human activity, is considered one of its main limitations, but it has the potential to be widely reduced by training courses (organized by dedicated scientific societies) and continuous "hands-on" practice. In the multi-disciplinary management of patients with ischemic stroke, neurological clinical skills are essential to "ask" neurosonology proper relevant questions, whose "answers" are expected to integrate and guide diagnostic and therapeutic work-up. A systematic presentation of such complex "dialogue" is beyond the aim of this chapter (to this purpose, see references [1, 2]); here, we focus on emerging pearls (and pitfalls) of neurosonology in two challenging

fields of applications: carotid stenosis and hyperacute revascularization. Of note, it would be better for neurologist and neurosonologist to coexist in the same professionist.

CAROTID STENOSIS: FROM DEGREE QUANTIFICATION TO PLAQUE CHARACTERIZATION

About one third of all ischemic stroke is due to large vessel atherosclerosis, in particular carotid stenosis, with high risk of early recurrence [3]. This is also true for cases with transient ischemic attack due to such disease [4]. Early carotid endarterectomy (CEA) proved greatly effective in reducing stroke risk in selected patients with symptomatic carotid stenosis ranging from 50 to 99% [5-7]. On these premises, after answering the questions “Has the patient suffered an ischemic stroke or transient ischemic attack? In which cerebrovascular district?”, neurologist should search for the etiopathogenesis of the event to “tailor” proper secondary prevention. As for potential selection for early CEA, neurologist should ask neurosonologist: “Has the patient a carotid stenosis? What is the degree of stenosis? May I trust you or should I ask radiologist another imaging technique such as CT/MR angiography or digital subtractive angiography?” These questions have been recently addressed by von Reutern *et al.*, who summarized the internationally available experience with ultrasonic grading of carotid stenosis [8].

Assessment of Plaque Morphology and Haemodynamic Effect

Pathogenesis of stroke due to carotid plaque relies on both hemodynamic effects (attributable to area reduction, not diameter reduction) and a complicated structure/surface eventually leading to emboli or occlusion. Diameter reduction alone is insufficient to evaluate the degree of stenosis, especially irregular ones, so that Doppler ultrasound and X-ray (or MR, CT) angiography each measure different biological variables and total agreement is impossible. Here, we focus on degree evaluation; advanced plaque characterization by ultrasound (e.g., plaque composition, plaque vascularization, thickness of the cap, plaque ulceration, and plaque motion) will be discussed in a further section.

Ultrasound B-mode accurately detects arterial wall thickness and minor plaques, providing multi-plane images of the wall itself and not only from the blood column. In case of severe stenosis, cross-sectional B-mode images are more difficult to generate because of shadowing and other artifacts; the same is true for color flow. Therefore, the more severe a stenosis, the more hemodynamic criteria are prevailing. The relationship between area and diameter reduction depends on the type of stenosis: in eccentric plaques diameter and area reduction are similar, while in concentric ones percentage area reduction is higher than the measured diameter reduction [9]; kidney-shaped or irregular plaques deserve a more complex evaluation. Despite these limitations, diameter reduction as shown by X-ray angiography (with “distal method” or “local method”) is considered to be the gold standard for decision-making because it was used respectively in NASCET and ECST trials. The “distal method” (NASCET) calculates the ratio of the minimal residual diameter of the stenosed segment to the diameter of a distal, clearly non-diseased segment of the internal carotid artery. It

corresponds better to the hemodynamic effect and it is preferred by radiologists and in existing guidelines for clinical decisions; however, this method has a relevant limitation in cases of very severe stenosis, where poststenotic flow volume diminishes and in consequence the diameter of the distal segment “collapses”, leading to a lower calculated degree of stenosis [10]. The “local method” (ECST) calculates the ratio of the minimal residual diameter of the stenosed segment to the presumed former diameter of the same segment; it better-illustrates plaque burdening. Both NASCET and ECST types of measurement are feasible with B-mode imaging if it is possible to visualize the residual lumen of the stenosis and the lumen of the poststenotic segment; Color flow imaging alone is less reliable for taking diameter or area measurements because of the influence of the gain setting.

Arterial narrowing leads to locally increased velocities; a hemodynamic effect is reached when pressure and flow volume are diminished in the poststenotic segment. When this effect is present, there is a raised risk of ischemia associated to a complicated plaque structure prone to embolism and/or insufficient blood supply. Flow velocity (in particular, peak systolic velocity, PSV) measured by means of Doppler sonography correlates with the narrowing measured in area reduction, as shown by the “Spencer’s curve.” [11]. However, when compared to X-ray angiography, degree of stenosis determined through PSV show considerable scattering of results [12-16], so that Duplex sonography has not been accepted as the sole methodology for definitive diagnosis by the American Heart Association [17]. Such disagreement can be explained by several reasons:

- First, there is the morphology of the stenosis as discussed above (area versus diameter; irregularities ill-represented).
- Second, there is ambiguity of the “Spencer’s curve,” with the possibility of the same velocity in a moderate stenosis and a nearly occluded artery; mimics of near occlusion include occlusion, intracranial disease, severe stenosis and even no stenosis [18].
- Third, measuring the angle of insonation needed for converting recorded Doppler frequencies into velocities may be subjected to errors due to disturbed flow (with stream lines differing from the vessel course) at the site of stenosis [19].
- Fourth, there is the influence of collateral flow. In a hemodynamically significant stenosis, the higher the capacity of intracranial collateral network (via the circle of Willis and, less effectively, via the ophthalmic artery, which differ from patient to patient), the less the poststenotic pressure decrease and, consequently, the intrastenotic velocity [20]. The same holds true for the PSV contralateral to an internal carotid artery occlusion [21].
- Fifth, there is the technical problems of spectrum analysis. At the site of stenosis, the Doppler spectrum is generated by both low-frequency components (due to vortices and flow separation) and high-frequency ones (due to jet), but the relative weight of the latter can be so low that it’s difficult to detect without special high-pass filtering or setting of the gain, leading to underestimation of the PSV [22].

Considering all these limitations, PSV as a single simplified diagnostic parameter appears insufficient. However, further ultrasound criteria (such as post stenotic PSV, collateral flow, end-diastolic flow velocity in the stenosis, “carotid ratio” – that is ratio of internal to common carotid PSV) allow to decide whether a measured PSV represents a less or more severe

stenosis within the scatter range. In particular, according to a multiparametric approach, carotid stenosis could be classified as follows:

- Low-degree stenosis 0% to 40% (NASCET). Velocity measurement rules out a more severe stenosis (e.g., PSV < 125 cm/second). B-mode imaging (in the longitudinal and cross-sectional planes) allows plaque detection and measures of reduction of diameter in percent, the thickness and length of the plaque as well as the residual lumen. Collateral flow is not present and carotid ratio is <2.
- Moderate stenosis 50% to 70% (NASCET). Local increase of velocity (e.g., 125 < PSV < 230 cm/second) and B-mode imaging can be combined for grading; collateral flow is not present, carotid ratio is >2 and end-diastolic flow velocity in the stenosis is <100 cm/second.
- Hemodynamically relevant stenosis >70% (NASCET) and occlusion. This is the domain of combined hemodynamic criteria: PSV > 230 cm/second; carotid ratio is >4 and end-diastolic flow velocity in the stenosis is >100 cm/second (except for 90% stenosis). Collateral flow may be demonstrated by examining the ophthalmic artery branches (omolateral external-internal artery supply), the anterior cerebral artery (proving cross-flow from contralateral internal carotid artery) or the P1 segment of the posterior cerebral artery (indicating collateral flow through the posterior communicating artery). Poststenotic flow velocity in the segment distal to the disturbed flow field is another useful criterion: if it is <50 cm/second the stenosis is about 80%, if it is <30 cm/second stenosis is about 90%. Color flow imaging is important as a guide for velocity measurement and, in addition to B-mode, is essential for differentiating occlusion from stenosis.

With a refined multiparametric ultrasound approach, used by skilled and certified operators, neurosonology for degree quantification of carotid stenosis warrants further re-evaluation in the guidelines regarding the management of such patients.

Assessment of Plaque Vulnerability

Moreover, ultrasound techniques have the potential to evaluate plaque vulnerability, allowing detection of cases at high risk of cerebrovascular ischemic events, which deserve aggressive diagnostic and therapeutic work-up. At present, only cases with recently symptomatic, moderate-to-severe carotid stenosis have a clear indication to early carotid endarterectomy; however, such indication could be extended to patient with “cryptogenic” TIA/stroke in the anterior circulation and moderate carotid stenosis due to a vulnerable plaque [23]. Furthermore, indications for CEA (with its related cost-effectiveness) in asymptomatic patients is currently under debate [24], mainly because medical therapy has greatly improved since ACAS trial, which randomized patients to CEA vs best medical therapy (supporting CEA) about 20 years ago [25]; in this context, neurosonology may help selecting cases with vulnerable plaque, which could be “truly good” candidates for CEA.

To better elucidate such issue, a systematic review and meta-analysis of ultrasound characteristics of symptomatic carotid plaques has been recently published by Brinjikji et al. [26]. First, authors defined ultrasound features of interest as follows:

- Heterogeneous echotexture: mixture of hypoechoic, isoechoic and hyperechoic lesions and/or heterogeneity in Gray-Scale-Median scores with mixture of hyperechoic, hypoechoic and isoechoic lesions
- Intraplaque motion: presence of mobile components within the plaque or on the plaque surface
- Irregular surface: irregular plaque surface with recesses less than 2 mm in depth
- Plaque echolucency: Type I, uniformly anechoic or Type II predominantly hypoechoic or anechoic with less than 50% echogenic areas. Or Gray-Scale-Median score below 30.
- Plaque neovascularity: plaque enhancement with contrast ultrasound imaging or areas of intraplaque Doppler or power Doppler signal consistent with neovascularity
- Ulceration: plaque surface crater measuring 2 mm or more or concavity with an echogenic line at the plaque base
- Complex plaque: any one of the above imaging features

After that, Authors screened and identified over one thousands published manuscripts (using MEDLINE and EMBASE) and included 23 papers with usable information in the final analysis. Ultrasound plaque characteristics with a higher prevalence in individuals with symptomatic compared to asymptomatic carotid artery stenosis included plaque neovascularity (OR = 19.68, 95% CI = 3.14 – 123.16), complex plaque (OR = 5.12, 95% CI = 3.42–7.67), plaque ulceration (OR = 3.58, 95% CI = 1.66 – 7.71), plaque echolucency (OR = 3.99, 95% CI = 3.06 – 5.19) and intraplaque motion (OR = 1.57, 95% CI = 1.02 – 2.41). Variables not associated with symptom status included heterogenous echotexture (OR = 2.68, 95% CI = 0.56 – 12.80) and surface irregularity without ulceration (OR = 2.38, 95% CI = 0.70 – 8.11).

In addition to direct plaque evaluation at the cervical level, ultrasounds can depict plaque instability through transcranial Doppler monitoring of microembolic signals (MESs) on ipsilateral middle cerebral artery. MESs detected in patients with carotid artery disease are due to unstable plaque and rapidly diminish following CEA [27]. In a systematic review of the literature, Ritter et al. found that 43% of patients with symptomatic carotid stenosis had MESs on TCD compared with just 10% of asymptomatic patients [28]. Furthermore, presence of just 1 MES was associated with 7.5 times higher odds of future symptomatic events in symptomatic patients and 13.4 times higher odds of embolic event in asymptomatic patients. Conversely, absence of MESs is associated with a very low risk of future symptoms in patients with asymptomatic carotid plaques. [29, 30].

In conclusion, ultrasound can usefully integrate other imaging modalities (X-ray angiography, CTA, MRA, PET) to evaluate degree of stenosis and plaque vulnerability [31].

HYPERACUTE REVASCULARIZATION: FROM DETECTION OF OCCLUSION TO SONOTHROMBOLYSIS

Until recently, intravenous tissue plasminogen activator (tPA) administered within 4.5 hours after symptom onset has been the only reperfusion therapy with proven efficacy in patients with acute ischemic stroke [32]. Pathogenesis of stroke is mainly due to arterial

occlusion and the aim of tPA is to achieve early vessel recanalization, saving ischemic penumbra and avoiding stroke-related long-term disability [33]; essential selection criteria for intravenous thrombolysis don't include vessel imaging, but determination of site of occlusion and monitoring of recanalization have allowed relevant advancements in stroke therapy.

For instance, intravenous tPA appears to be much less effective at opening proximal occlusions of the major intracranial arteries (which account for more than one third of cases of acute anterior-circulation stroke) [34], leading to poor prognosis [35]. Recent trials [36-40] have shown that, in selected patients with stroke due to proximal intracranial arterial occlusion in the anterior circulation, endovascular treatment (on top of standard therapy) significantly improves patients' outcome. On these premises, neuroimaging and endovascular therapy have to be considered key-elements in the current management of patients with hyperacute stroke; here, we focus on the diagnostic and therapeutic role of ultrasounds, which may usefully integrate such armamentarium.

Diagnosis and Monitoring: The TIBI Score

Grading and characterization of extracranial carotid stenosis have been discussed in the previous section. Ultrasound evaluation of hypoplasia, stenosis and occlusion in the vertebrobasilar system is feasible too [41]. In the hyperacute setting, assessment of intracranial arteries is crucial; Demchuk et al. developed a Thrombolysis in Brain Ischemia (TIBI) classification by using transcranial Doppler (TCD) to detect and monitor intracranial vessel residual flow signals in middle cerebral artery and basilar artery [42]:

- Grade 0: absent. Absent flow signals are defined by the lack of regular pulsatile flow signals despite varying degrees of background noise.
- Grade 1: minimal. Systolic spikes of variable velocity and duration; absent diastolic flow during all cardiac cycles based on a visual interpretation of periods of no flow during end diastole; reverberating flow is a type of minimal flow.
- Grade 2: blunted. Flattened systolic flow acceleration of variable duration compared to control; positive end diastolic velocity and pulsatility index <1.2.
- Grade 3: dampened. Normal systolic flow acceleration; positive end diastolic velocity; decreased mean flow velocities (MFV) by >30% compared to control.
- Grade 4: stenotic. MFV of >80 cm/s and velocity difference of >30% compared to control side; if both affected and comparison sides have MFV <80 cm/s, due to low end-diastolic velocities, MFV >30% compared to the control side and signs of turbulence. Of note, criteria for intracranial stenosis (>50%) assessment through transcranial color-coded duplex (TCCD) sonography had been published by Baumgartner et al. in 1999 [43].
- Grade 5: normal. <30% mean velocity difference compared to control; similar waveform shapes compared to control.

In the same manuscript, Demchuk et al. reported about 109 patients undergoing intravenous thrombolysis, finding that pre-tPA National Institute of Health Stroke Scale (NIHSS) score were higher in patients with TIBI grade 0 than TIBI grade 4 or 5 flow [42]. Moreover, TIBI flow improvement to grade 4 or 5 occurred in 35% of patients with an initial

grade of 0 or 1 and in 52% with initial grade 2 or 3; the 24-hour NIHSS scores were higher in follow-up in patients with TIBI grade 0 or 1 than those with TIBI grade 4 or 5 flow. TIBI flow recovery correlated with NIHSS score improvement; on the other hand, lack of flow recovery predicted clinical worsening or no improvement. In-hospital mortality was 71% for patients with posterior circulation occlusions; it was 22% for patients with pre-tPA TIBI 0 or 1 compared with 5% for those with pre-tPA TIBI 2 or 3 anterior circulation occlusions [42].

Such findings were confirmed by Saqqur et al., who reported that clinical response to thrombolysis was influenced by the site of occlusion: patients with no detectable residual flow signals (as well as those with terminal internal carotid artery occlusions) resulted least likely to respond early or long term [44].

FROM DIAGNOSIS TO TREATMENT: SONOTHROMBOLYSIS

Delivery of tPA to and through the thrombus is likely dependent on minuscule residual red blood cell flow around the thrombus and plasma flow through the thrombus [45]. Mechanical agitation can expose shallow layers of thrombi to circulating tPA and facilitate streaming of plasma through the thrombus, thus bringing more tPA to binding sites. Ultrasound is a pressure wave that can travel through tissues and deliver this mechanical momentum to stagnant flow areas and clot interfaces. Several experiments have confirmed the ability of ultrasound to enhance fibrinolysis through disaggregation of non-cross-linked fibrin strands, further thinning them, and promoting plasma flow and lytic drug delivery to the clot [46-48]. Kilohertz frequencies were thought to induce more mechanical stretching, whereas megahertz ultrasound promotes fibrinolysis more through enzymatic mechanisms [49, 50]. In 2000 Alexandrov et al. serendipitously discovered as part of the clinical routine that stroke patients with intracranial arterial occlusion treated with systemic tPA and monitored with 2-MHz transcranial Doppler (TCD) could recanalize early during treatment and experienced dramatic clinical recovery. This observation was subsequently confirmed in a randomized controlled trial called CLOTBUST (Combined Lysis of Thrombus in Brain ischemia using transcranial Ultrasound and Systemic tPA) [51]: among 126 enrolled patients with stroke due to middle cerebral artery occlusion (63 patients in each group), 83% achieved any recanalization (46% complete, 27% partial) with tPA and TCD versus 50% (17% complete, 33% partial) with tPA alone within 2 hours of treatment ($P < 0.001$). Sustained complete recanalization at 2 hours was 38% versus 13%, respectively ($P = 0.03$).

In the last 15 years, several groups have tested ultrasound with tPA (“sonothrombolysis”) or ultrasound without tPA (“sonolysis”, in patients with contraindications to tPA) according to different protocols, varying in duration (from one to two hours), frequency (TCD with 2.0 MHz; TCCD with 1.8 MHz; low frequency, non-diagnostic ultrasound of 300 KHz) and use of microbubbles (which have the potential to enhance the mechanical action of ultrasound). Of note, TRUMBI trial using low frequency ultrasound (300 kHz) was prematurely interrupted for safety reasons because the authors observed an excess of intracranial haemorrhages in the treated group in comparison with control patients [52]. As for site of occlusion, middle cerebral artery (whose monitoring is feasible through simple probe-supporting helmets, too) was the most studied vessel, while very small data exist on patients with internal carotid artery terminus or basilar artery occlusion, whose natural history lead to

a very bad prognosis [53, 54]. The results of principal published studies have been reviewed in the last few years by Tsivgoulis et al., Ricci et al., Saqqur et al. 2014 [55-57]. Among these, the manuscript by Ricci et al. included only randomized controlled trials [58-62], with a total of 233 patients. As for main results, Authors reported that:

- For the primary outcome (death or dependency at three months), five studies with a total number of 206 patients were available (four defined independence as a modified Rankin score of 0 to 2 and one used 0 to 1). Patients treated with sonothrombolysis were less likely to be dead or disabled at three months (odds ratio (OR) 0.50, 95% confidence interval (CI) 0.27 to 0.91).
- For the secondary outcomes, failure to recanalise was lower in the sonothrombolysis group (230 patients) (OR 0.28, 95% CI 0.16 to 0.50), no significant difference was found in mortality (206 patients) and in cerebral haemorrhage (233 patients).

In conclusion, sonothrombolysis appeared to reduce death or dependency at three months (although CIs were quite wide), and increased recanalisation without clear hazard; however, a larger clinical trial was warranted. To this purpose, an Italian randomized multicentric pilot study called “ULTRAS” (Ultrasound Thrombolysis in Acute ischemic Stroke) is on-going.

Moreover, potential future development of sonothrombolysis include engineering of microbubbles (such as fibrin-targeted microbubbles, tPA loaded liposomes and immune-particles) [63-65], intra-arterial administration of ultrasound (through EKOS catheter [66]) or microbubbles [67], hands-free transcranial Doppler device [68]. Taken together, current evidence and future perspectives suggest that ultrasound may usefully integrate therapy of patients with hyperacute ischemic stroke.

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Chapter 28

INTRAVENOUS THROMBOLYSIS: PRESENT EVIDENCES AND FUTURE PERSPECTIVES

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ABSTRACT

Intravenous thrombolysis is a widely established therapy for the hyperacute management of ischemic stroke and has been increasingly recognised over the years as the crucial treatment to reduce stroke disability and mortality. The progressive diffusion of Stroke Units, the raising awareness about cerebrovascular disease and the revision of inclusion and exclusion criteria, led to a larger amount of patient to benefit from thrombolytic therapy. Several trials are ongoing to enhance the efficacy and safety of thrombolytic agents and to grant to a larger amount of patient to be efficaciously treated. This chapter describes the studies that led to the approval of use of the recombinant tissue plasminogen activator for the treatment of acute stroke and past and current trials with newer thrombolytic agents and advanced diagnostics and therapeutic techniques.

Keywords: intravenous thrombolysis, stroke, stroke unit, alteplase, tenecteplase, desmoteplase

INTRODUCTION

Since its introduction in late '90s, intravenous thrombolysis (IV TL) with Alteplase played a crucial role in the therapy for acute ischemic stroke in patient admitted to Stroke Units and was increasingly established all around the world. Several indications and contraindications to therapy changed during the years, especially with a progressive enlargement of the therapeutic window that, together with a widespread diffusion of Stroke

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Units, led to a larger amount of patients to be eligible for the treatment. Results from several randomized controlled trials (RCTs) led to the approval of use of the recombinant tissue plasminogen activator (rt-PA, *alteplase*) within 4,5 hours from symptoms onset on November 2010 in Europe by the *European Medicines Agency* (EMA).

INTRAVENOUS THROMBOLYSIS

Clot dissolving drugs produce their effect by restoring the blood flow through the hypoperfused tissues in the so-called ischemic penumbra, where a potentially salvageable area suffer from the reduction of blood supply. However, since the brain cells rapidly die from the time the ischemic injury occurs, reperfusion therapy may be more efficacious the earlier they are administered.

The first attempts to treat stroke patients with clot dissolving drugs were carried out in the 90s with *Streptokinase*, a polypeptide derived from the β -haemolytic *Streptococcus*, and *Urokinase*, a human derived enzyme, but they showed an excessive increase in symptomatic intracranial haemorrhage (sICH). Newer generation thrombolytic agents with shorter half-life and more fibrin-specificity (*alteplase*, *pro-urokinase*, *reteplase*, *tenecteplase*, *desmoteplase*) were later evaluated but only alteplase has been licensed for use within 4.5 hours of stroke in the US, Canada and Europe.

Result of several RCTs, the *National Institute of Neurological Disorders and Stroke* (NINDS) *study group trial* [1], the *European Cooperative Acute Stroke study I* (ECASS) [2] and *II* (ECASS II) [3], the *Alteplase ThromboLysis for Acute Noninterventional Therapy in Ischaemic Stroke* (ATLANTIS) *study* [4], and their pooled analysis [5] showed that IV TL with rt-PA, despite a greater incidence of hemorrhagic transformation, is a highly effective treatment in selected patients with acute ischemic stroke.

Basing on this data, in September 2002, the EMA released a conditional approval for use of rt-PA at a dose of 0.9 mg per kilogram body weight with a maximum of 90 mg, infused intravenously over 60 minutes, with 10% of the total dose administered as an initial bolus. As requested by the EMA, stroke patients treated with rt-PA were recruited in the post-marketing observational *Safety Implementation of Thrombolysis in Stroke-Monitoring Study* (SITS-MOST) with the aim to verify the safety and efficacy profile of IV TL in a “real world” routine clinical practice.

Begun on December 2002 and completed on April 2006, the SITS-MOST study treated 6483 patients in 285 centres among 14 European countries. Symptomatic hemorrhagic infarction (sICH), defined as a clinical worsening of at least 1 point on the NIHSS scale in the presence of any type of intracerebral bleeding, occurred in 7.3% (95% CI 6,7-7,9) of patients, compared with 8,6% (95% CI 6,3-11,6) of the previous RCTs; the mortality rate at 3 months amount to a total of 11,3% (95% CI 10,5-12,1) against 17.3% (95% CI 14,1-21,1) in RCTs. The efficacy of treatment, evaluated on patients who reach a modified Rankin scale (mRS) score of 0-2 at 3 months, was achieved by 54.8% (95% CI 53,5-56,0) compared with 49.0% (95% CI 44,4-53,6) in RCTs [6]. Therefore, SITS-MOST results largely confirmed those of RCTs and a final approval of rt-PA within the 3 hours window was released by EMA in 2006.

A review of SITS-MOST showed a substantial undertreatment compared to the numbers of potentially eligible patients, mainly because acute stroke patients arrived at the treatment

hospitals beyond the therapeutic window. Same figures were reported in the US [7] where the potential number of treated patients would be increased tenfold with strategies to reduce the door to needle time [8]. Regarding to this, it is noteworthy that by the end of the SITS-MOST study, the recruitment of new centres, clearly not experienced in IV TL, increased by 50% without any substantial modifications in treatment safety and efficacy. Hence, the importance of efforts to implement the Stroke Units, together with strategies adopted to extend the therapeutic window. The meta-analysis of the individual data of ATLANTIS, ECASS I-II, and NINDS trials showed an efficacy of IV TL until 4,5 hours from the symptoms onset, despite a time related reduction of the effect [5]. Hence, as an adjunctive request by the EMA, a new randomized placebo-controlled trial, the *European Cooperative Acute Stroke study III* (ECASS III), was done to verify the efficacy and safety of IV TL versus placebo in the 3 to 4,5 hours therapeutic window. Patients treated with alteplase reached a more favourable outcome than those in placebo group (52,4% vs. 45,2%, adjusted OR of favourable outcome - mRS 0/1: 1,42, 95% CI 1,02-1,98) even with a higher incidence of sICH (7,9% vs. 3,5% - OR 2,38, 95% CI 1,25-4,52). However, mortality did not differ significantly between the two groups (7,7% vs. 8,4% - OR 0,90 95% CI 0,54-1,49) [9]. At the same time, based on “real life” data from the SITS International Stroke Registry (SITS-ISTR), results from the comparison of 664 patients receiving IV TL between 3 and 4,5 hours and those treated within the 3 hours window, confirmed the same efficacy and safety of rt-PA between the two groups for any outcome measures. A functional independence was achieved by 58,0% and 56,3% of patients respectively (aOR 0,93 95% CI 0,84-1,03), with an incidence of sICH of 8,0% vs. 7,3% (aOR 1,13 95% CI 0,97-1,32) and mortality rate of 12,7% vs. 12,2% (aOR 1,15 95% CI 1,00-1,33). Such encouraging results, led to the approval of the extension to 4,5 hours of the therapeutic window for alteplase by EMA in 2010.

Over the same time, more than 3000 ischemic stroke patients were randomized to rt-PA or control in the *International Stroke Trial 3* (IST 3) within the 6 hours windows, regardless of age or severity of neurological deficits. Basing on the uncertainty principle, a large amount of patients not fulfilling EMA license criteria for the use of rt-PA (95%) was randomized. At 6 months, a functional independence was achieved by 37% of treated patients and 35% of controls (OR 1,13 95% CI 0,95-1,35), ICH occurred in 7% vs. 1% respectively, while mortality was similar in both groups (27%). Moreover, the treatment within 3 hours showed its greater efficacy in over-80 patients and in those with more severe stroke at onset [10].

Results from a further 2014 pooled analysis of individual data of 6756 patients from all the published RCTs with alteplase, showed that IV TL within 4,5 hours was associated with an increase of the odds of a good outcome at 3 to 6 months, irrespective of stroke severity at onset. Moreover, a clear correlation between the onset-to-treatment (OTT) interval and efficacy of rt-PA treatment was found (OTT $\leq$ 3 hours: OR 1,75 95% CI 1,35-2,27; OTT 3-4,5 hours: OR 1,26 95% CI 1,05-1,51; OTT >4,5 hours: OR 1,15 95% CI 0,95-1,40), highlighting the overwhelming importance of an early treatment in stroke patients. The analysis also showed an increase in the odds of sICH in rt-PA treatment group (PH2 definition: 6,8% vs. 1,3% OR 5,55 95% CI 4,01-7,70; 7 day fatal ICH 2,7% vs. 0,4% OR 7,14 95% CI 3,98-12,79), and its significant correlation with stroke severity, while similar mortality rates was found between the two groups (HR 1,11 95% CI 0,99-1,25) [11].

A large international double-blind trial is ongoing to randomize patients to rt-PA treatment between 4.5 and 9 hours after symptom onset or with unknown time-window: the *European Cooperative Acute Stroke Study-IV* (ECASS IV). Patients are selected via Magnetic

Resonance (MR) evidence of a penumbral mismatch defined as a perfusion volume (perfusion weighted image, PWI) to infarct core (diffusion weighted image, DWI) ratio of ≥ 1.2 , and a minimum perfusion lesion volume of 20 ml and followed up at 3 months [12].

NEWER THROMBOLYTIC AGENTS: DESMOTEPLEASE, TENECTEPLASE

The thrombolytic agent from vampire bat saliva, *desmoteplase*, was tested in several trials of acute ischemic stroke with a longer therapeutic window, due to a lower neurotoxicity and consequently lower incidence of hemorrhagic complications, compared with rt-PA. The first trial, the Desmoteplase in Acute Ischemic Stroke Study (DIAS), treated 104 patients within 3 to 9 hours after stroke onset based on mismatch criteria on PWI/DWI MR imaging [13]. The study demonstrated that IV desmoteplase administration was associated with a better clinical outcome compared with placebo, with low sICH rate. Remarkably, patients treated within 6-9 hours had same chances for a good clinical outcome as patients treated in the 3 to 6 hours time window. The subsequent *Dose Escalation of Desmoteplase in Acute Stroke* (DEDAS) trial confirmed that treatment with IV desmoteplase 3 to 9 hours after ischemic stroke onset appeared safe and improved clinical outcome in patients fulfilling MR mismatch criteria [14].

Unfortunately, the following two studies did not confirm the good results of previous ones. The DIAS II, investigating the efficacy of 2 doses of desmoteplase in stroke patients with a distinct ischemic penumbra of at least 20%, found no significant differences between the groups in clinical response rates, while mortality rate was higher in the highest-dose group. The DIAS-III/IV recruited patients with a high-grade stenosis or occlusion in proximal cerebral arteries supplying the infarct area, treated within 3-9 hours after the symptoms onset. The trial was stopped prematurely due to futility analysis.

Among the newer thrombolytic drug, *tenecteplase* (TNK), a modified recombinant tissue plasminogen activator molecule, showed promising as a potentially safer alternative to alteplase. IV TL with rt-PA was compared with different doses of TNK in two, phase 3, RCTs [15, 16], subsequently included in a Cochrane review [17] where no significant statistical differences emerged from the 2 studies (187 pts) about the death and dependency outcome measure. Moreover, the trial of Haley and colleagues [16] included a higher dose TNK group (0.4 mg/kg) was discontinued because of safety concerns about incidence of sICH at that dose, whereas imaging selection criteria in the trial of Parsons and colleagues [15] resulted in exclusion of a large amount of patients who were otherwise eligible for rt-PA. Hence, the *Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis* (ATTEST) [18], compared the outcome of IV TL with TNK (0.25 mg/kg maximum 25 mg, 52 pts) versus rt-PA (standard dosage, 52 pts) within 4.5 h of stroke onset, in patients not selected on the basis of advanced neuroimaging. No significant differences were noted in terms of percentage of penumbral tissue salvaged, incidence of sICH, total ICH events or incidence of serious adverse events between the TNK and rt-PA groups.

The previously mentioned 2013 Cochrane review [17] also compared different outcome measures for thrombolytic treatment with rt-PA, urokinase, desmoteplase and TNK at different drug dosages but failed to found a superiority of a drug to another. Result from 7 studies with 730 patients showed no differences for the death and dependency outcome

measure, while non-conclusive evidences that higher doses correlate with higher rate of fatal ICH were found analysing 1274 patients in 10 studies. A RCT is ongoing with the aim to compare the standard rt-PA dosage (0.9 mg/Kg) with a low-dose (0.6 mg/Kg). This part of the *Enhanced Control of Hypertension and Thrombolysis Stroke Study* (ENCHANTED) [19] finished its recruitment on 17 August 2015 with more than 3300 patients randomized.

THROMBOLYSIS IN THE ELDERLY

Among the efforts to implement thrombolytic treatment in acute ischemic stroke, one must consider the enlargement of IV TL to older patients. Due to the progressively increasing burden of common risk factors such as dyslipidemia, hypertension, cardiovascular disease and diabetes during the age, cerebrovascular diseases tend to be more and more common in older population. Unfortunately, patients aged over 80 years old had been poorly represented in first clinical trials due to their highest comorbidity rate and presumed poorer outcome. Older patients have the highest absolute risk of death following a stroke and are more likely to require long-term care than younger ones. A first survey on IV TL in over 80 showed a higher mortality compared to younger patients, with lower rate of favourable outcome, despite the same rate of sICH [20]. Data from the NINDS trial [1] suggested that younger patients benefit from thrombolytic treatment more than older ones when adjusted for stroke severity and the multivariable analysis of outcome predictors in the SITS-MOST study and pooled RCTs found that older age was related to poor outcome [21]. However, further results emerged from the large cohort of over 80 patients in the IST 3 (53% of the total) where the benefit of treatment in this group was, actually, as large as in younger patients [10]. Similar results was found in a large subsequent pooled analysis, with an amount of 1729 of over 80 patients (26% of the total), where age was shown not to change the effect of alteplase on odds of a good outcome nor on incidence of fatal ICH [11].

INFLUENCE OF GENDER

Even if the natural history of stroke showed that women have a worse outcome than men, early works suggested that they could benefit from a better response to thrombolytic treatment with higher recanalization rate and better functional outcome [22, 23]. The analysis of baseline characteristics of women from the SITS-MOST study showed that they were older, had a higher NIHSS score at baseline and were more likely to have a history of arterial hypertension and atrial fibrillation compared to men, whereas the latter were more frequently affected by hyperlipidemia and diabetes mellitus and had a smoke habitus. No differences were found in the 3-month favourable outcome after corrections for confounders, confirming that IV TL may modify the outcome favouring women as compared to the natural course of stroke. Male sex actually resulted independently associated with a higher odds ratio for sICH and mortality [24].

NEURO-IMAGING TECHNIQUES CONTRIBUTION

Advanced neuro-imaging techniques could improve the efficacy and safety of IV TL, extending the therapeutic window and leading to a more accurate selection of patients. Brain computed tomography and MR, with perfusion and angiographic study, and Ultrasound techniques have given interesting results. MR can suggest useful prognostic information about safety and efficacy of IV TL when patients arrive beyond the 3 hours time window or when exhaustive anamnestic information is lacking. This could be of extreme utility for those patients with wake up stroke or for those aphasics and living alone, when the onset of symptoms could not be determined at all. A potentially salvageable brain tissue could be found in more than 80% of stroke patients in the 3 to 6 hour time interval and an eventual recanalization could be strongly influenced by the promptness of treatment [25-27]. The *Diffusion and perfusion imaging Evaluation For Understanding Stroke Evolution* (DEFUSE) study showed that baseline MR findings could identify subgroups that are likely to benefit from IV TL 3 to 6 hours after symptoms onset, based on mismatch profile and extent of DWI area [28]. Moreover, in the *Echoplanar Imaging Thrombolytic Evaluation Trial* (EPITHET) the site of arterial obstruction was found to be a strong predictor of outcome after rt-PA administration in the 3 to 6 hour interval [29]. Based on MRA data, the study showed that internal carotid artery (ICA) obstruction carries a poor prognosis, whereas good outcomes are associated with middle cerebral artery obstruction. A subsequent analysis of both studies suggested that the infarct growth attenuation after rt-PA over placebo was greater in patients with baseline arterial obstruction than those without [30].

Proximal intracranial occlusion has thought to be a target for ultrasound-enhanced thrombolysis. Ultrasound techniques are not only fast and reliable non-invasive methods to assess regional blood flow but, when combined with thrombolytic therapy, they showed to improve recanalization of occluded vessels. A meta-analysis of 6 randomized and 3 nonrandomized clinical studies of several *sonothrombolysis* methods suggested, in 2010, that ultrasound and rt-PA increase the likelihood of recanalization compared to IV TL alone, without a raised risk of sICH [31]. A subsequent Cochrane revision, 2 years later, evaluated 206 patients from 5 studies finding a net benefit of sonothrombolysis in reducing death and disability and in determining an early recanalization [32]. Same figures were found analysing the 191 patients randomized at IV TL alone or at sonothrombolysis plus IV TL. Moreover, a subanalysis of one of the evaluated studies, the CLOTBUST trial, showed a higher efficacy of sonothrombolysis in patients with occlusion of major intracranial vessels [33]. However, due to the small number of patients and trials heterogeneity, recommendations for the use of sonothrombolysis in clinical practice cannot be done. A recent RCT to evaluate the safety and efficacy of transcranial ultrasound, using an operator-independent headframe, as an adjunctive therapy to rt-PA treatment in subjects with acute ischemic stroke (CLOTBUSTER) has been terminated on April 2015 [34].

THROMBOLYSIS IN SPECIAL CONDITIONS

The efficacy of thrombolytic treatment for acute strokes with uncommon aetiology or special situations such as ICA occlusion, cerebral venous and dural sinus thrombosis, cervical artery dissection and pregnancy, has also been considered.

The efficacy and safety of IV TL in patients with acute stroke due to ICA occlusion was studied in the *Systemic Thrombolysis in Patients With Acute Ischemic Stroke and Internal Carotid ARtery Occlusion* (ICARO) study [35]. The comparison of patients with acute ischemic stroke and ICA occlusion treated within 4,5 hours (253 pts) to matched non treated patients (253 pts), showed that thrombolytic therapy increases the chances to reach a favourable outcome, even though it leads to higher death and intracranial bleeding rates. The association with ICA occlusion and adverse outcome was confirmed in a subsequent analysis of consecutive patients treated with IV TL, divided into those with (137 pts) and those without ICA occlusion (1761 pts). Death and death or disability rates at 90 days were found to be higher in ICA occlusion group, even after logistic regression analysis [36].

Ischemic strokes in the posterior circulation account approximately from 11% to 16% of all ischemic strokes and have been suggested to have different etiopathogenetic, clinical and outcome characteristics. Recent unpublished data shown that IV TL in patients with posterior circulation stroke (291 pts, 12%) compared to those with anterior circulation stroke (2163 pts) lead to similar mortality rates and favourable outcome chances. Moreover, vertebrobasilar strokes independently predicted lower risk of sICH after IV TL, but only according to the less severe NINDS definition.

A particular and threatening condition is that of basilar artery occlusion (BAO), presenting high mortality rates. A systematic review in patients with acute BAO, comparing endovenous and endovascular treatment, showed unfavourable results in term of mortality and disability with low rates of good outcome in both treatment groups [37]. Similar findings emerged from the *Basilar Artery International Cooperation Study* (BASICS) where patients with BAO were treated mainly with intra-arterial therapy or IV TL or antithrombotic treatment only: high rates of unfavourable outcome (mRS 4-6: 69%) and mortality (39%) were found, and no unequivocal superiority of one treatment on the others could be established [38-40].

The use of IV TL in patients with cerebral venous and dural sinus thrombosis could be considered in deteriorating cases despite anticoagulant therapy, but only few data are available. A systematic review of study on cerebral vein and dural sinus thrombosis found that a good outcome was reached in 22 of 25 patients treated with IV TL (rt-PA used only in 2 patients, 7,7%), even if a significant proportion of bleedings was reported [41].

The safety and efficacy of IV TL in dissection-related ischemic stroke was investigated in a recent prospective study coupled with comprehensive meta-analysis results. Data from a total of 39 stroke patients with cervical artery dissection suggested that thrombolysis is safe and functional recovery rates favourably compared with the overall IV TL efficacy reported in phase III trials [42].

Pregnancy was historically regarded as a contraindication to IV TL even if rt-PA is too large a molecule to cross the placenta and is not known to be teratogenic. To date, suggestions from literature are only limited to several small published case series and it is also debated whether IV TL or endovascular treatment be more appropriate in pregnancy. Major potential risks to mother and foetus are, aside from mother intracerebral haemorrhage, major uterine or foetus haemorrhage or spontaneous abortion. However they seem to be reasonably low in pregnancy and must be balanced against the potential of a disabled outcome without treatment. Hence, pregnancy should not be considered as an absolute contraindication to IV TL and the decision should be dependent on individual factors such as time from symptom

onset, imaging findings, other concomitant medical conditions or feasibility of an endovascular thrombectomy [43, 44].

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Chapter 29

HAEMORRHAGE: EMERGING THERAPIES

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ABSTRACT

Primary cerebral hemorrhage (ICH) is a major public health problem associated with a mortality rate of 40% in the first 30 days and case-disability rate until 80%. Clinical approaches for ICH varies from the aggressive surgery to the supportive medical care alone. Medical management of ICH should include controls of both blood pressure and intracranial pressure (ICP), osmotherapy, fever and glycemic controls, seizure therapy and multidisciplinary care in a specialized Stroke Unit. Overall, 15% of ICH are associated with anticoagulation use and in those, reverse therapy is recommended after monitoring the anticoagulation activity. Surgical therapy after ICH is effective in reducing mass effect, improving intracranial pressure and brain perfusion, preventing dangerous compartment shifts, as well as stopping the cascade responsible for secondary injury. in randomized trials there has been no reported advantage from surgery. However, Didecompressive hemicraniectomy (DHC) associated to haematoma evacuation and minimally invasive surgery could be useful in patients with ICH.

Keywords: cerebral hemorrhage, clinical management, reverse therapy, surgical therapy

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INTRODUCTION: CAUSES AND NATURAL HISTORY

Primary cerebral hemorrhage (ICH) is a devastating disease with no established treatment and is therefore regarded as a major public health problem, giving rise to 10 to 15% of strokes in western countries and up to 30% in Asia [1]. ICH, described as spontaneous extravasation of blood into the brain parenchyma, is associated with a mortality rate of 40% and case-disability rates have been reported to reach 80%. ICH is classified as either primary or secondary. Hypertension is the most common (~65%) cause of spontaneous ICH, with other major causes being: amyloid angiopathy, brain tumors, aneurysms, arteriovenous malformations, cerebral cavernous malformations and arteriovenous fistulae [1]. ICH associated with the use of anticoagulants constitutes almost 15%, with a 7- to 10-fold increase in the risk of developing ICH from baseline [2]. Overall, 30% of ICHs are associated with antiplatelet use. ICH is classified into ganglionic (putamen, caudate and thalamus), lobar, cerebellar and pontine. The basal ganglia are affected in 40-50% of patients, cerebrum 20-50%, thalamus 10-15%, cerebellum 15% and brainstem less than 10% [3]. Their locations are correlated with outcomes and potential treatments and only the surgical decompression for cerebellar haemorrhages is widely accepted. Moreover, intraventricular bleeding has been reported to lead to poor prognosis. ICH prognosis is also associated to haemorrhagic volume, with half of all deaths occurring in the acute phase, especially in the first 48 h. Very large haemorrhages (>100 ml) are associated with a very poor prognosis [4]. Brain injury, mass effect and rim of edema are known to induce severe neurological deficits and death. Edema tends to increase rapidly after ICH, with a peak in the second week after stroke. The region around a hematoma is characterized by edema, apoptosis, necrosis, and inflammatory cells, which often leads to a secondary injury. Moreover, mass effect and physical disruption associated with the primary lesion, the response of the body/tissue to the hematoma (e.g., inflammation) and the release of clot components (e.g., haemoglobin/iron) may worsen the secondary injury [1]. These event phases could be targets for the treatment of ICH, which will be discussed in this chapter.

TREATMENT

No therapy has been reported to improved outcome in primary cerebral hemorrhage (ICH). Clinical approach for ICH varies from aggressive surgery to supportive medical care alone. Medical management of ICH should include controls of both blood pressure and intracranial pressure (ICP), osmotherapy, fever and glycemic controls, seizure therapy and multidisciplinary care in a specialized Stroke Unit or neurological intensive care unit (ICU); admission to the former seems to improve outcomes, while long stays in ICU seem to predict worse outcomes. Multidisciplinary team-care, together with more aggressive care seem to provide greater benefit to patients with ICH [5].

Medical Treatment

Elevated blood pressure (BP) is a common feature of acute ICH patients due to stress, pain, increased ICP, and premorbid acute or persistent elevations in BP. In fact, high BP has

been associated with greater hematoma expansion, neurological deterioration as well as higher rates of death and dependency after ICH [6]. For impaired cerebral blood flow autoregulation, excessive blood pressure reduction may exacerbate ischemia in the area surrounding hematoma and worsen neurological impairment. The American Heart Association Guidelines recommend aggressive infusion of antihypertensives including urapidil, labetalol, esmolol or nicardipine whenever systolic blood pressure (SBP) on admission exceeds 220 mmHg. While, for patients with SBP between 150 and 220 mm Hg on admission and without contraindications to acute BP treatment, an acute lowering of SBP to 140 mm Hg has been recommended by the INTERACT II study results [6]. Oral therapy is recommended for all patients regardless of BP, 24-72 h after infusion therapy or when a clinical condition has been stabilized [7].

Monitoring of ICP is recommended when an ICH patient is comatose (Glasgow Coma scale score <9), using external ventricular drain or fiber optic intraparenchymal control. Two other approaches for reducing ICP include positioning the head at a 30° angle and hyperventilation.

The phase III FAST trial (fVIIa for Acute Hemorrhagic Stroke Treatment) investigated for the efficacy of fVIIa in patients with ICH treated within 3 h of symptom onset. Of the 841 patients included in the study, 268 received placebo, 276 received 20 µg/kg, and 297 received 80 µg/kg of fVIIa; at 3 months the combined rates of death and disability were 24%, 26% and 29%, respectively. Mortality was similar among the groups. The rate of arterial thrombosis resulted higher in the group treated with 80 µg/kg of fVIIa (28%) compared to the placebo (18%) or 20 µg/kg of fVIIa (17%) groups. Thus, fVIIa did not lead to better functional outcomes, despite it being associated with a significant reduction in the rate of hematoma expansion [8]. The FAST trial subgroup analysis indicated possible benefit for patients under 70 years of age, those with baseline hematoma volumes less than 60 ml, those with baseline intraventricular hemorrhage volumes less than 5 ml and those with time from onset less than or equal to 2-5 h at admission [8].

Regarding the use of anti-edema, the administration of mannitol for 3-4 days in patients with ICH and neurological deterioration associated with high intracranial pressure or mass-effect, has been reported to improve functional recovery. Whereas, two randomized trials have reported no benefit from the regular use of intravenous mannitol boluses [9, 10].

Early clinical seizures (within 1 week), most often occur in young patients after ICH and they have been reported to be as high as 16%, with most occurring at or near onset. Clinical seizures should be treated with antiepileptic therapy, but prophylactic anti-seizure medication is not recommended [6].

Medical Care and Management of Complications

In a review of 1,421 patients with ICH, care limitations or withdrawal of life-sustaining interventions were the most common (68%) causes of death [11]. Airway management could be necessary in patients with ICH, but its benefit is generally offset by not being able to perform a thorough neurological examination; 10% of intensively treated patients will need the tracheostomy treatment for airway support, and early use of this approach might reduce the risk of aspiration and long-term mechanical ventilation.

About 30% of patients with ICH have stress gastric hemorrhages. Prophylactic H₂ blockers or proton pump inhibitor drugs are useful in decreasing the numbers of gastric events [12]. Moreover, in the first 2 weeks after onset, deep-venous thrombosis can be detected by ultrasonography in 20-40% of patients with pulmonary embolism risk. For these patients, low-molecular-weight heparin can be started on the second day after onset of intracerebral hemorrhage when the patients are neurologically stable. To this regard, a recent meta-analysis, including 1000 patients with acute hemorrhagic, comparing unfractionated heparin or low-molecular-weight heparin or heparinoids with treatments other than anticoagulants (elastic stockings, intermittent pneumatic compression or placebo), reported that early heparin prophylaxis for venous thromboembolism is associated with a significant reduction in pulmonary embolism, a non-significant reduction in deep venous thrombosis or death, and a non-significant increase in hematoma enlargement. In the studies included in this meta-analysis, anticoagulant therapy was started 24 h to 6 days from stroke onset [13]. Intermittent pneumatic compression (IPC) is an alternative treatment according to the results of Clots in Legs Or sTockings after Stroke (CLOTS) 3 trial. In this randomized controlled study, including 1,438 patients admitted to hospital within 3 days of acute stroke, IPC was both an effective and inexpensive method for reducing the risk of deep venous thrombosis and improving survival in immobile stroke patients [14]. However, only about 10% of the patients, had an intracranial hemorrhage in this study.

In the acute period of ICH, temperature and glucose controls with antipyretic medication and insulin infusion, are recommended due to the fact that hyperthermia and hyperglycemia both have neurotoxic effects.

Surgical Treatment

The surgical therapy after ICH is effective in reducing mass effect, improving intracranial pressure and brain perfusion, preventing dangerous compartment shifts, as well as stopping the cascade responsible for secondary injury [15]. In order to do so, general anesthesia and invasive craniotomy followed by dissection through viable brain to reach the hematoma need to be carried out. There is no standard protocol for hematoma removal and guidelines regarding evacuation are lacking.

The STICH I trial, which included 1,033 patients with both lobar and deep hemispheric ICH randomized to early surgery (within 96 hours of ictus) versus medical management with delayed surgery, reported a 10% relative increase in good outcomes in the surgical arm, but no benefit in 6-month favorable outcome (26%) compared with the medical arm (24%, $P = 0.41$) using the Glasgow Outcome Scale score. However, in a pre-specified subgroup analysis, subjects with lobar ICH ≤ 1 cm from the brain surface who underwent surgery had an 8% absolute increase in good outcomes compared with similar subjects in the medical arm [16]. This subgroup was subsequently investigated in STICH II [17]. In this randomized clinical trial, 601 patients with lobar hematomas of 10 to 100 mL located ≤ 1 cm from the brain surface and without intraventricular hemorrhage or coma were randomized to surgery versus initial medical management; 307 patients received surgery at a median of 26 hours after stroke onset. There was no advantage with surgery, because 41% of patients in the surgical group had a favorable outcome compared to 38% of patients in medical group. [17]. However, there was a trend toward lower 6-month mortality in surgical group compared to

medical group [15]. Furthermore, the STICH II authors performed a meta-analysis on 2,266 patients, observing an advantage for surgery therapy, but data were heterogeneous [17]. When cerebellar hemorrhages are greater than 3 cm, or when brainstem compression or hydrocephalus is suspected, surgery has been recommended by several non-randomized studies.

Open surgery to date, has failed to significantly improve the outcome of ICH patients, whereas less invasive approaches including microsurgery are now under evaluation in clinical trials.

INTRACEREBRAL HAEMORRHAGE DURING ANTICOAGULANT THERAPY

Worldwide, 15% of ICHs are associated to anticoagulation use. In fact, it has been reported that patients under anticoagulation treatment have a five-fold to ten-fold increased risk of ICH compared to the general population [18].

Antagonists

Hematoma expansion occurs in about 30% of patients with spontaneous ICH, but in up to 54% of patients with ICH associated with vitamin K antagonists. Use of warfarin increases the risk of progressing bleeding and mortality in patients with ICH. Being so, reverse therapy is recommended, even though a Canadian multicenter registry study has reported that both mortality and morbidity remained high [19]. For several decades, vitamin K alone was employed with the limitation that reversal therapy required more than 24 hours of therapy to fully reverse anticoagulation, even with intravenous administration at onset. In fact, the reversal effect of vitamin K alone begins around 2 hours after administration and reaches its maximum at 24 hours when liver function is normal [20].

The multicenter randomized trial 4F-PCC studied the use of prothrombin-complex concentrate (PCCs/Beriflex) versus fresh frozen plasma (FFP) for warfarin reversal in patients with ICH taking an oral vitamin K antagonist (INR > 2). The study reported the superiority of 4F-PCC over FFP regarding rapid INR reversal with the safety profiles being similar [21]. The ongoing FFP Versus PCC in Intracranial Hemorrhage trial is exploring the efficacy of these agents in spontaneous hemorrhagic stroke (NCT02429453).

In clinical practice, FFP, PCC or vitamin K are employed to reverse ICH in patients taking an oral vitamin K antagonist (INR > 2) [22-24]. However, the administration of FFP requires thawing and cross matching, carries a risk of allergic, infectious [6], and transfusion reactions, and often requires large volumes for full INR correction [6]. PCC on the other hand, does not require cross matching, can be reconstituted and administered rapidly in small volumes (20-40 mL), and has been successfully processed for inactive infection agents. Its principal limitation is that it can increase the risk of thrombotic events [21].

Being so, The European Heart Rhythm Association recommends that tranexamic acid, desmopressin or activated factor VII could be considered [25].

Direct Oral Anticoagulants (DOACS)

In the three large randomized trials on direct oral anticoagulants (DOACS), ARISTOTLE, RE-LY and ROCKET-AF [26], oral anticoagulants were reported to be associated with a lower risk of ICH, when compared to warfarin. However, the issue of avoiding hematoma growth, when patients with ICH are treated with DOACS has yet to be addressed adequately. Specifically, validated quantitative assays for monitoring the anticoagulant effect of DOACS need to be developed [27].

Monitoring the Anticoagulant Activity of DOACS

Normally, monitoring tests are not required for patients under treatment with DOACs. However, they could be useful in patients with ICH and stroke or those requiring emergency surgery [28]. In fact, the standard coagulation assays (aPTT, PT, TT), that are used to monitor heparin derivatives or vitamin K anticoagulants, may not accurately reflect the precise anticoagulant activity of DOACs [29]. Yet, these tests could be useful in clinical practice. Moreover, the activated specifically thromboplastin time (aPTT) is more responsive to dabigatran and an aPTT level two-fold greater than normal range 12 to 24 hour post-dose; which could suggest the presence of a dabigatran effect. Furthermore, aPTT test has high inter-individual and reagent variability, in fact 18% of patients taking dabigatran 150 mg twice daily have been reported to have a normal aPTT at trough [30]. The thrombin time (TT) is normal when no or minimal plasma dabigatran levels are present. A prolonged TT can be used to indicate the presence of dabigatran, but not its concentration, as the test has excessive sensitivity to dabigatran concentration. Dabigatran plasma concentration can be evaluated by Hemoclot thrombin inhibitors, ecarin clotting time and ecarin chromogenic assay [31]. Therapeutic levels of apixaban have shown essentially no effect on APTT and little effect on PT assays, whereas, rivaroxaban and edoxaban often elevate both the aPTT and the prothrombin time (PT) [31]. PT may provide a qualitative assessment for the presence of FXa inhibitors, but this data should be taken with caution [32]. Furthermore, anti-FXa assay might suggest anticoagulation effect absence [27]. To this regard, in an emergency setting, ex recommend, checking aPTT for dabigatran and PT for rivaroxaban and apixaban is recommended [33]. Concerning FEIBA it contains the vitamin-K dependent the factors II, IX, X, activated VII factor and protein C in non-activated form and reduces bleeding in dabigatran treated rats, but lower doses of FEIBA could reverse anticoagulant effect of rivaroxaban too [34]. Moreover, A study was conducted on 12 healthy volunteers receiving rivaroxaban (20 mg b.i.d) or dabigatran (150 mg b.i.d) for 2.5 days, followed by a bolus of 50 IU/kg of PCC, where the latter reversed the prolonged prothrombin time (PT) caused by rivaroxaban, but PT was not prolonged by dabigatran. Only the use of rFVIIa and FEIBA have been proposed after studies *in vitro* to reverse the anticoagulant effect of apixaban [27].

Dabigatran Reversal Agents

Antibodies to dabigatran (Idaruciximab) have been recently developed providing reverse effects within 1 minute of i.v. bolus injection in animal models and has structures comparable

thrombin, high affinities (350 times) for dabigatran and no activities in coagulation tests or platelet aggregation [35]. Idarucizumab administration in the absence of dabigatran has no reported effect on coagulation parameters or endogenous thrombin potential, suggesting that it is unlikely to be prothrombotic. The phase III trial REVERSE AD (NCT02104947) where idarucizumab was administered to patients with major bleeding or requiring emergency surgery, reported that it normalized both direct thrombin time and ecarin clotting time within minutes and was well-tolerated [36]. Moreover, the administration of intravenous fluids, the induction of diuresis and dialysis should be considered for patients on dabigatran, but not for those on apixaban or rivaroxaban.

Rivaroxaban Reversal Agents

PRT06445 (Portola compound or factor andexanet) is a truncated form of enzymatically inactive factor Xa that can reverse any of the Xa inhibitors [37]. In a phase 3 trial, andexanet immediately neutralized the anti-Xa activity of rivaroxaban in healthy elderly volunteers and resulted being well tolerated [38]. Another molecule under clinical development for reverse rivaroxaban activity is BAY1110262.

Apixaban and Edoxaban Reversal Agents

Andexanet reversal of apixaban with an IV bolus dose followed by a continuous infusion was administered to healthy elderly volunteers who were already taking 5 mg of apixaban twice daily [38]. No serious adverse or thrombotic events were reported.

PER 997 (Ciranparatag) is a small molecule that as been reported to reverse the anticoagulant effects of edoxaban, apixaban, dabigatran, rivaroxaban as well as unfractionated and low molecular weight heparin *in vitro* and in rats. As of 2016 Phase 3 trials are underway 2016 [39].

THERAPEUTIC STRATEGIES UNDERWAY

The effects of BP-lowering have been explored in two recent randomized clinical trials, ATACH II and INTERACT 2. The latter trial studied 2,839 patients with acute ICH and SBP between 150 and 220 mm Hg within 6 hours of ICH. Overall, 719/1,382 patients (52.0%) receiving intensive treatment (SBP target of <140 mm Hg within 1 hour of randomization and for 7 days, following protocols that included locally available intravenous agents) were compared with 785/1,412 patients (55.6%) receiving standard treatment (SBP <180 mm Hg). No significant effect of intensive BP-lowering treatment was observed regarding hematoma growth, but a modest benefit was seen in functional recovery in the BP-lowering treatment group [6]. ATACH II is now investigating the therapeutic benefit of the intensive treatment vs. standard treatment including death and disability (mRS 4-6) at 3 months in ICH patients treated 0-4.5 hours from symptom onset (NCT01176565).

A neuroprotective approach has been studied in animal models and results have indicated a decrease in brain edema after reducing the releases of iron, carbon monoxide or biliverdin from lysed red blood cell [40]. These results need to be confirmed in human trials.

The NXY-059 trial investigated the benefit from a 72-hour infusion of placebo or intravenous NXY-059, a free radical scavenger, within 6 hours of stroke onset in 607 ICH patients. The drug, when compared to placebo, lead to slightly less hematoma increase, but there were no observed effects on mortality or functional outcome in the patients taken the drug [41]. Additionally, the prior use of statins has been reported to have benefit in recent retrospective cohort studies [42]. Moreover, the principle trials on open surgery (craniotomy) in patients with ICH, both STICH I and II, have reported inconclusive results.

Regarding surgery, decompressive hemicraniectomy (DHC) is a neuro-surgical treatment that aims to decrease intracranial pressure (ICP) and is generally indicated for patients with ischemic or hemorrhagic stroke or those with traumatic brain lesions [43]. However, clinical benefits have been observed only for malignant middle cerebral artery infarction [44]. Experimental studies on animal models with ICH have observed that DHC reduces mortality, yet results regarding neurological outcomes have been controversial [45]. Regarding human studies, a recent review of literature including 191 patients with ICH volumes more than 60 ml, aged 40-60 years, and Glasgow Coma Scale scores less than 8 before surgery, observed favorable outcomes in 41% and average mortality of 21% [45] following DHC and hematoma evacuation. Another recent study, including a 51 patients series, undergoing a combination of DHC and hematoma evacuation, observed a significant midline-shift and an improved outcomes only in lobar ICH patients [46]. Limited data are available regarding clinical benefit from DHC without hematoma evacuation. In fact, studies to date have been small and often retrospective case series or case-control studies have been performed [42]. The SWITCH trial is currently comparing the best medical treatments in intracerebral hemorrhage against decompressive hemicraniectomy (NCT02258919). However, other surgical approaches are being explored as alternative for craniotomy. In view of the fact that MISTIE II and other pilot trials of minimally invasive surgery have shown to improve patient outcome, a phase III trial using this approach was started in late 2013. Minimally invasive surgery over conventional surgery includes some advantages: reduced operative time, possibility of local anesthesia and reduced local surgical trauma. Endoscopic aspiration has been suggested as a possible treatment for superficial supra-tentorial ICH, as it been shown to reduce mortality, especially in younger patients [47].

Finally, regarding thrombolysis, small studies have investigated on the benefits from, urokinase or tissue plasminogen activator (t-PA) in the treatment of intraventricular hemorrhage (IVH). Currently, the Phase III Clear IVH Trial is aiming to evaluate the efficacy and the safety of r-tPA in treating IVH (NCT00650858). Trials have assessed Factor VIIa versus placebo. STOP-IT is now evaluating the predictive value of spot-sign in the growth of ICH, and whether factor VIIa improve functional outcomes compared to placebo in these patients. Another ongoing trial is SPOTLIGHT (NCT01359202), which has the same aims and includes 15 Canadian centers.

Table 1. Assays and recommendations for reversal options of anticoagulants

	Half-Life (hours)	Monitoring Assay	Effective Reversal Strategies	Non-effective Reversal Strategies
Warfarin	40	PT	FPP, PCC, Vitamin K [6], Tranexamic acid, desmopressin, activated factor VII [24]	
Dabigatran	14-17	aPTT, TT, ECT, Hematoclot [25], ecarin-chemogenic assay	induction of diuresis, Dialysis, FEIBA [25], PER977 [48]	Vitamin K PCC rFVIIa
Rivaroxaban Edoxaban	5-9	Anti-FXa, PT, aPTT PiCT [26]	PCC, FEIBA [5], PER977 [45], PRT06445 (Lu G 2013).	rFVIIa Vitamin K Dialysis
Apixaban	9-14	Anti-FXa, mPT, HepTest [25]	rFVIIa, FEIBA [25], PER977 [45], PRT06445 [37].	Vitamin K Dialysis

PT: prothrombin time; aPTT: activated partial thromboplastin time; TT: thrombin time; ECT: ecarin clotting time; mPT: modified prothrombin time; PiCT: prothrombinase-induced clotting time; HEPTEST: heparin clotting test; PCC: prothrombin-complex concentrate.

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Chapter 30

MANAGEMENT OF INTRACRANIAL STENOSIS

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ABSTRACT

Intracranial arterial stenosis (IAS), most often attributable to primary atherosclerosis, is the most common cause of stroke worldwide and is also associated with a high rate of recurrent stroke, even in patients receiving the best medical treatment. Severity of stenosis is the strongest predictor of stroke. The most commonly used therapeutic strategies for IAS include medical therapy, identification and control of risk factors and intervention to prevent thromboembolism and restore blood flow. High-resolution MRI, indirect revascularization technique (EDAS), precondition treatment and therapy with direct oral anticoagulants (DOACS) have been reported to provide benefits in both diagnosis and treatment of IAS. Future studies need to stratify large numbers of patients with IAS into subgroups according to the risk of recurrence and complications to deliver better treatment.

Keywords: intracranial stenosis, management, endovascular treatment

INTRODUCTION

Intracranial arterial stenosis (IAS) has been observed in 20-40% of patients with atherosclerotic disease and is associated with a risk of ipsilateral stroke of up to 7.6%

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annually. The currently available management options include medical treatment, risk factor interventions and surgical or intravascular treatments. The benefits from these options remain unclear [1].

EPIDEMIOLOGY AND PATHOLOGY

IAS is most often attributable to primary atherosclerosis, with other rare causes including embolic events, arterial dissection, vasculitis, infections, exposure to radiation, sickle cell disease and Moya-Moya disease [2]. IAS prevalence, evaluated by transcranial doppler ultrasound in asymptomatic Caucasian populations has been recorded to be 13%, whereas autopsy studies on the same groups have reported rates of up to 80% in those older than 80 years [3]. IAS has been primarily observed in Asian, Hispanic, Black and Indian populations, as well as in patients with vascular risk factors; suggesting racial and ethnic differences could be found in genetic susceptibility and lifestyle [4]. The reported proportion of patients with symptomatic intracranial stenosis among those hospitalized for ischemic cerebral was reported to be up to 50% in Asian populations [5]. Worldwide, IAS is the most common cause of stroke [6]: 10% of all reported strokes in USA [4] and 33-50% of all reported strokes in Asia [7]. Further, IAS is associated with a high rate of recurrent stroke, even in patients receiving the best medical treatment (higher in Blacks and women); the risk of stroke and death with IAS is disproportionally elevated in women, reported to be up to 29% [3]. Epidemiological studies have reported that Blacks with IAS have both an increased incidence and mortality from stroke, as well as stroke recurrence at an earlier age [8]. The WASID study reported that women had higher risks for both recurrent ischemic stroke and the combined end-point of stroke and vascular death, leading to an 85% higher risk of ischemic stroke for women when compared to men.

IAS is commonly hypothesized to be the result of a multifactorial process involving several risk factors leading to endothelial injury, lipid deposition on the arterial walls and inflammation. Metabolic syndrome, diabetes mellitus, abdominal obesity, hypertension and smoke are risk factors for endothelium injury and arterial atherosclerotic plaque. Multiple mechanisms alone or combined have been documented to be involved in the onset of ischemic stroke in patients with IAS: downstream ischemia, artery-to-artery emboli, local thrombosis and perforator vessel occlusion caused by plaque. The severity of stenosis is the strongest predictor of stroke [9]. In fact, the WASID study reported that severe stenosis $\geq 70\%$, was associated with a significantly higher subsequent risk of stroke in the territory of a symptomatic intracranial stenotic artery, compared to stenosis $< 70\%$; while it appeared to decline when stenosis was between 90% and 99% [9]. Also the presence or not of neurological symptoms has been correlated with different stroke risks: the rate of ischemic stroke has been reported to be 12% for symptomatic stenosis versus 3.5% for asymptomatic stenosis, at one year follow-up [10-11].

THERAPEUTIC STRATEGIES

There are Three commonly used therapeutic strategies for IAS: medical therapy, identification and control of risk factors and intervention to prevent thromboembolism and restore blood flow.

MEDICAL THERAPY

From 1955 until recently, the results from several retrospective studies had suggested that warfarin was more effective than aspirin in preventing progression of the disease and decreasing mortality, especially in the vertebral-basilar system. [12]. In 2005 the results of the WASID trial reported that when comparing aspirin (1200 mg/day) to warfarin in patients with symptomatic IAS over 1.8 years of follow-up, there was no observed difference between the two study arms for incidence of recurrent stroke, whereas warfarin was seen to increase both major hemorrhages and death. Furthermore, subgroups of patients with higher risk, such as those with severe stenosis (70-99%), vertebro-basilar stenosis and previous stroke symptoms was reported had no significant benefits from warfarin therapy.

In the CLAIR study (Clopidogrel Plus Aspirin for Infarction Reduction Study) [13], patients with IAS treated with the combination of aspirin and clopidogrel had lower rates of micro-embolic signals detected by transcranial Doppler until day 7, compared to patients receiving aspirin alone. The short-term use of aspirin plus clopidogrel, followed by aspirin alone, was also examined in the SAMMPRIS study. The study reported an overall 30 day rate of stroke or death equal to 5.8%. In the WASID study, patients with angiographically verified 50 to 99 percent stenosis of a major intracranial artery received warfarin or aspirin and the rate of stroke or death was observed as being 10.7% during a median follow-up of 14.7 months was recorded.

The WASID and SAMMPRIS trials also reported high annual rates of recurrent stroke in patients with IAS despite medical treatment: 15% [10] and 12.2%, respectively [1].

The Clopidogrel in High-risk patients with Acute Non-disabling Cerebrovascular Events (CHANCE) trial reported that the combined treatment of clopidogrel plus aspirin decreased the 90-day risk of stroke without increasing hemorrhage when compared to aspirin alone in patients with TIA or minor stroke [14]. Overall, 1,089 patients with MRA images available in CHANCE were included in a sub-analysis on the efficacy and safety of clopidogrel plus aspirin vs aspirin alone and found that 608 patients (55.8%) had IAS. In fact, patients with IAS had higher rates of recurrent stroke (12.5% vs 5.4%; $p < 0.0001$) at 90 days compared to those without IAS. There were no statistically significant differences observed between patients with and without IAS regarding primary outcome of any stroke (hazard ratio for clopidogrel plus aspirin vs aspirin alone: 0.79 [0.47-1.32] vs 1.12 [0.56-2.25]; interaction $p = 0.522$) and any bleeding event (interaction $p = 0.277$) [15].

The phosphodiesterase inhibitor cilostazol together with aspirin was seen to decrease the progression of intracranial stenosis, as measured by MRA and TCD at 6 months [16]. Cilostazol and clopidogrel were compared in a follow-up study on 457 patients with symptomatic middle cerebral or basilar artery and the number of new events (ischemic or hemorrhagic) at 7 months was similar between the two groups [17].

RISK FACTOR CONTROLS

Modifiable vascular risk factors for IAS include hypertension, smoking, diabetes, high total cholesterol concentration, low high-density lipoprotein cholesterol concentration and metabolic syndrome, while non-modifiable factors include race, age, certain angiotensin-converting enzyme polymorphisms, increased plasma endostatin/vascular endothelial growth factors and certain glutathione S-transferase omega-1 gene polymorphisms [18]. The WASID and SAMMPRIS trials examined the use of ACE inhibitors to decrease blood pressure and the use of statins to lower LDL-cholesterol concentrations. The WASID trial recommended maintaining blood pressure lower than 140 mmHg and cholesterol concentration inferior to 5-20 mmol/l [10]. In a trial on cilostazol in IAS (TOSS-2) [16] increased apolipoprotein B/A was associated with progression of stenosis, while increased HDL was associated with stable stenosis [19]. In the SAMMPRIS trial, the aggressive medical treatment arm, compared to the angioplasty and stenting arm, thoroughly monitored risk factors including blood pressure and LDL concentration. The identification and aggressive management of modifiable vascular risk factors is mandatory and systolic blood pressure should be kept ≤ 140 mmHg or ≤ 130 mmHg in patients with diabetes and low-density lipoprotein concentration should be maintained at <70 mg/dl [20-21].

SURGICAL TREATMENT

Limited surgical treatments have led to benefit in IAS. The International Cooperative Study of Extracranial to Intracranial (EC/IC) bypass trial, a prospective multicenter randomized trial, failed to report lower rates of stroke recurrence when comparing surgery to medical treatment in patients with extracranial occlusion or intracranial stenosis (EC/IC bypass Study Group 1985). The study enrolled 1,377 patients with extracranial carotid occlusion or intracranial carotid or middle cerebral artery stenosis, comparing the extracranial to intracranial bypass to aspirin treatment alone. Possible explanations for these study results could have been associated with the abrupt increase in the flow observed in the extracranial to intracranial bypass surgery, due to hypertension and hemorrhage and the competitive flow, which can trigger by the bypass which changes the flow and can lead creating stasis in the stenotic segment or adjacent segments [3]. The COSS trial compared extracranial-intracranial bypass to medical treatment, reporting that the 2-year stroke and death rate was not significantly better than that of the medical arm (21% versus 23%). The peri-procedural stroke rate in this trial was 15%, similar to that reported by the EC/IC bypass study. Bypass has been proposed for vertebro-basilar stenosis, but data regarding IAS cases are limited [22].

ENDOVASCULAR TREATMENT

In the 1980s endovascular treatment was considered a treatment option for intracranial stenosis, with its initial being promising [23]. Angioplasty alone resulted being complicated leading to a restenosis rate of up to 50% for treated patients and a high rate of vessel dissection [24]. For this, percutaneous angioplasty and stenting (PTAS) became the preferred

option for endovascular treatment, even though PTAS was known to have a high risk of peri-procedural stroke, due to the difficulty in navigating the intracranial vessels, trauma during balloon inflation and the deployment of the stents. In fact, PTAS was compared to aggressive medical treatment in patients with severe IAS in the SAMMPRIS trial, however the trial had to stopped prematurely (only 59% of planned patients had been enrolled) given that stroke or death occurred in 14% of patients in the angioplasty and stenting arm within the first 30 days, compared to 5.8% in the medical arm [1]. The 1-year rates of stroke and death were 20.0% for the PTAS arm and 12.2% for the medical arm, after a mean duration of follow-up of 11.9 months [1]. The SAMMPRIS trial results extinguished the enthusiasm for endovascular treatment in patients with IAS. The increased peri-procedural events were not due to operator inexperience [25], but instead more likely due to the elevated severity of the stenoses and/or the rigorous evaluation by local neurologists. Other studies have confirmed the results from SUMMPRIS [24]. In a review of literature on endovascular treatment in IAS, using the Wingspan stent system (self-expanding nitinol intracranial microstent) in association with the Gateway percutaneous transluminal angioplasty (PTA), it had already been tested in SAMMPRIS, which reported a combined risk of TIA and stroke of 6.2% for ipsilateral and a 9.5% risk for all territories [23]. Regarding possible risk factors, smoking habit was not significantly associated with peri-procedural stroke given that smoke habit increases the conversion of clopidogrel to an active metabolite. While, basilar artery stenosis, severity of stenosis, diabetes, older age and clopidogrel load were significantly associated to the presence of peri-procedural intracranial haemorrhages [26].

In-stent restenosis (ISR) is a serious complication, often symptomatic, observed in 3.3% of Gateway PTS balloon and Wingspan stent systems [23], caused by stimulation of intimal hyperplasia by the radial force of the stent. The Enterprise stent is a closed-cell intracranial self-expanding stent designed to reduce the incidence of in-stent restenosis, when compared to the Wingspan stent, it tends to reduce the radial force. A large series of patients treated with Enterprise stent reported the combined rate of neurological morbidity and death to be 7.7% at 30 days, while the rate of restenosis was in line with that observed for the Wingspan stent [27]. In coronary circulation, drug-eluting stents (DES) are commonly and successfully used to reduce the likelihood of restenosis, but single-center studies exploring DES application in intracranial stenosis have been conflicting [28-29]. The safety of DES in intracranial stenosis has not yet fully been established, due to repeatedly observed associations to high rates of technical failure and peri-procedural complications. Currently, the combined rate of morbidity and mortality is estimated to be 3.8% at 30 days [23]. Derived from the coronary approach, Balloon-Expandable stents (BESs), are also used in IAS: the combined rate of stroke and death at 30 days has been estimated at 23.9%, whereas the majority of these strokes were severe (13%) [23]. The most frequently observed complication is thromboembolism which can cause downstream vascular occlusion. Patients with stented basilar arteries have been reported to experience a high rate of perforator territory infarcts, [25]. As well, dissection of the vessel is frequent for anatomical reasons including the mobile position of cerebral artery in the subarachnoid space and the tortuous course of cerebral arteries. Other complications can include restenosis and vascular perforation as well as vessel rupture.

ALTERNATIVE PERSPECTIVES

Future studies are needed to stratify large numbers of patients with IAS into subgroups according to the risk of recurrence and complications in order to relize targeted therapeutic strategies. Specifically, high-resolution MRI, using 3 tesla or higher magnets, could be reccommandable to best suited for characterizing IAS, leading to a better description on the states of the stenosis, lumen and vessel walls; therein assisting in the prediction of any critical stenosis [30].

The indirect revascularization technique (EDAS), which involves the transposition of a segment of a scalp artery into the surface of the brain without a direct anastomosis, has been used in treating Moya-Moya disease. In comparison to direct revascularization, EDAS is less technically demanding, as it does not require the temporary occlusion of cerebral vessels and allows for a gradual development of revascularization only where the brain requires it. This surgical treatment has been tested in IAS with contrasting results [31].

Brief limb ischemia seems to confer systemic protection against myocardium infarction in humans and in animal models; emerging data suggest the same protection ("ischemic preconditioning") might be useful in stroke for cerebral tissue [32]. Meng et al. have reported a decrease in new events in a trial including 68 patients treated with brief repetitive bilateral arm ischemic preconditioning (occlusion of both brachial arteries with a blood pressure cuff twice daily for 300 days) [33].

Trials assessing benefit and safety from direct oral anticoagulants (DOACS) are underway following a prospective study reporting that treatment with acenocoumarol, adjusted to maintain internal normalized ratio (INR) between 2 and 3, decreases the risk of recurrence by reducing the progression of intracranial stenosis [34]. Moreover, oral anticoagulants may play an important role in preventing the deposition of new thrombotic material on the artery stenotic walls, however, according to WASID study, warfarin was seen to increase both major hemorrhages and death compared to aspirin. Therein, DOACS could be considered a safe alternative treatment to warfarin in reducing progression of IAS, given that they have been associated with a significant reduction in intracranial bleedings compared to warfarin use.

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Chapter 31

“TELESTROKE,” A FRONTIER IN THE GLOBALIZATION ERA

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ABSTRACT

Stroke telemedicine (or telestroke) allows the delivery of neurological expertise to remote locations with limited access to neurological care. Current telestroke networks are steadily growing, mainly to support emergency department consultations. These consultations are focused on the hyperacute treatment of stroke patients with rt-PA.

Most telestroke programs use a combination of interactive video conferencing and teleradiology to support the quality of their consultations. These telestroke networks have been demonstrated to increase the rate of thrombolytic treatment, reduce patient hospital stays and improve functional outcomes. From a macroeconomic perspective, telestroke is also cost efficient because the costs are overcompensated by the savings that result from the increased number of patients who are discharged home and the reduced costs of long-term care.

Despite convincing evidence of the effectiveness of telestroke, worldwide, there are limited options for reimbursement for telestroke networks, which is seen as a major barrier to the growth and sustainability of telestroke networks. Nevertheless, the last 12 years have seen the rise of telestroke networks, enabling access to state-of-the-art conservative acute stroke therapies regardless of geographical location.

Although teleneurology is currently mostly used for acute stroke treatment, it is likely that it will become incorporated into other aspects of neurological care, such as stroke telerehabilitation or prehospital telestroke.

This chapter reviews the development, current status, problems and perspectives of telestroke from a global perspective.

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INTRODUCTION

With the agricultural and industrial revolution in the late 19th century, the relatively stable equilibrium between rural and urban populations broke due to unprecedented growth in urban residents. Today, more than 50% of the world's population lives in cities.

In general, the range of offered services is much greater in urban regions compared to rural locations. This principle holds also true for the medical sector. Rural locations with a low number of inhabitants obtain their emergency medical services mostly at small country hospitals. These hospitals often have less than 100 beds and normally do not have a neurology department. Patients who cannot be treated in such hospitals are shipped to the next urban hospital that offers the required expertise.

Although this structural organization is economically and clinically reasonable, it is problematic for stroke patients. With the approval of intravenous recombinant tissue-type plasminogen activator (rt-PA) by the Federal Drug Administration in 1996, there is a potent treatment for acute ischemic stroke available, and it is beyond doubt that swift initiation of treatment is strongly beneficial to stroke patients [1, 2]. However, the American Heart Association estimates that less than 5% of all ischemic stroke patients in the USA are treated with rt-PA. They further reported that only approximately 50% of patients have access to primary stroke centers within a drive time of 60 min, and half of all hospitals have no staff neurologists [3, 4]. These are some of the reasons that cause a reduction in the application of rt-PA in small and rural hospitals. A recent study has estimated that the chance for a patient to receive rt-PA is tenfold less in a rural emergency department compared to an urban primary stroke center [4].

An analysis of the MEDPAR database revealed that from 2005-2007, 64% of US hospitals did not use rt-PA [5]. Some of these smaller hospitals used a “ship and drip” model, in which a patient with a suspected stroke is shipped to an urban hospital before receiving intravenous rt-PA (drip). This procedure wastes valuable time, and often, rt-PA cannot be administered in the urban hospital due to the now lapsed time window for rt-PA treatment.

Studies have identified pre-hospital as well as in-hospital barriers to the delivery of thrombolysis for acute stroke [6, 7]. One of the most important in-hospital barriers is physician uncertainty in administering rt-PA and concern about the adverse effects of rt-PA [6]. The uncertainty and concern is caused by the very complicated and restricted indications for rt-PA. There are 26 formally listed contraindications for rt-PA. However, it is insufficient to know these contraindications because there are a multitude of studies available that have investigated these contraindications as well as their importance and risk characteristics. Each of these contraindications has to be weighed for each individual patient with respect to his/her risk-benefit ratio. Moreover, the recently published intervention studies of additional or (in the case of specific contraindications) alternative methods make the decision even more complicated [8-11]. However, the decision for or against thrombolytic therapy has to be made quickly because the therapeutic effect of rt-PA is highly time dependent. Incorrect decisions

can cause severe adverse effects and even the death of the patient. The natural reflex of most physicians is that it is better to do nothing than to do something wrong.

Accordingly, stroke patients are in need of acute neurological stroke expertise in local hospitals. This need is targeted by telestroke networks. By way of modern communication technologies, these networks bring stroke expertise to rural locations when it is needed.

However, there are also some problems with telestroke, such as reimbursement for the use of telestroke networks, licensing and accreditation issues as well as technological problems.

This chapter reviews the development, current status, problems and perspectives of telestroke from a global perspective.

HISTORY

The Beginning of Telemedicine

The idea of delivering health care through telecommunication is so obvious that telemedicine has effectively existed as long as telecommunication. As an early precursor, African villages used smoke signals in cases of serious disease to warn people to stay away from that village. The invention of the telephone in the nineteenth century marked the beginning of modern telemedicine. In 1906, the first electrocardiogram trace was transmitted using telephone cables. A further example is the Australian outback, where people used 2-way radios to communicate with the Australian Royal Flying Doctor Service in the 1930s. With the rise of radiological imaging, telemedicine became more technically demanding. The earliest reference in the medical literature describing the transmission of radiological images was published in 1950 [12]. This was called “Telognosis,” which was a combination of the three terms “teleo roentgen diagnosis.” Although this term did not achieve mainstream use, it marked the beginning of remote medical image transmission. The term “Telemedicine” was not introduced in the medical literature until the study of R.G. Mark was published in 1972 [13]. During the next decade, Canadian radiologists at Montreal’s Jean-Talon hospital created a teleradiology system [14].

A major influence on the development of telemedicine originated from developments in the manned space-flight program. Physicians at the National Aeronautics and Space Administration (NASA) were concerned about the effects of zero gravity on astronauts. To monitor the physiological functions of the astronauts, sophisticated biomedical telecommunication systems were developed [15]. Moreover, plans needed to be developed in case of a medical emergency. Such an emergency in space parallels emergencies in rural locations on Earth. In both cases, people with significant health problems have difficulty accessing appropriate health care. Within the space program, telemedicine was further developed within the STARPAHC (Space Technology Applied to Rural Papago Advanced Health Care) program that was sponsored by NASA and designed by Lockheed Missiles and Space Corporation during the 1970s. The program used the most advanced technology to explore the possibilities of providing improved health care to a remote population in southern Arizona via telecommunication [16-17].

In addition to the space program, the development of television was the second major influence on telemedicine at this time. In the year 1959, a neurological examination was transmitted by the use of two-way interactive television to students at the University of Nebraska [18]. This technology was further developed and applied to medical consultations in 1964. A two-way television link was established between the University of Nebraska and Norfolk State Hospital to provide case consultations, medical examinations and speech therapy [14]. In the late 1960s and early 1970s, multiple telemedicine projects were funded by the US federal government to determine the feasibility and capabilities of telemedicine systems. These systems were further developed in the next decades and were applied in multiple medical disciplines.

The Development of Telestroke

Within the discipline of neurology, telemedicine has received increasing attention after the Food and Drug Administration approved the use of rt-PA for acute stroke in 1996. However, the indications for this drug were (and are) difficult, and many contraindications have to be considered. This has led to a divergence in the use of rt-PA between specialized urban stroke centers and small rural hospitals. An analysis of the MEDPAR database revealed that between 2005-2007, 64% of US hospitals did not use rt-PA [5]. Some of these smaller hospitals used a “ship and drip” model, in which a patient with a suspected stroke is shipped to an urban hospital before receiving intravenous rt-PA (drip). This procedure wastes valuable time, and often, rt-PA cannot be administered in the urban hospital due to the now exceeded time window for rt-PA treatment.

Due to these problems, the use of telemedicine for the hyperacute treatment of stroke patients with rt-PA was discussed [19]. In 1997, the University of Iowa received funding from the National Library of Medicine to develop telemedicine for stroke in rural areas [19, 20]. Additionally, several states were awarded grants by the Department of Health and Human Services (HHS) over the years 1997-1999 for several different rural pilot telemedicine projects.

The first telehealth applications dedicated to treat stroke patients in remote hospitals were developed by the Department of Neurology of the Medical College of Georgia in 2001 [21-23]. This development led to the launch of the REACH system in 2003, which enabled the examination of patients at rural hospitals [21-23]. The REACH system consisted of a mobile cart that was located at the rural emergency department. It captured video images and data that were transferred to the hub hospital in Georgia via an internet connection. The data transfer was compliant with Health Information Portability and Accountability Act requirements. This system enabled the performance of NIHSS evaluations and the review of the brain imaging [21]. Over the first two years, this system was used for 194 acute stroke consults, of which 30 patients received rt-PA. This pilot study demonstrated that the use of rt-PA in rural community-based hospitals was possible. The door-to-needle time improved from 98 minutes in the first year to 78 minutes in the second year. In subsequent years, multiple other technical solutions dedicated to the requirements of telestroke networks were developed.

Over the last decade, the use of telestroke systems has increased. A Canadian survey in 2006 identified 12 telestroke networks [24], while in 2009, the American Heart Association

found 33 organizations that provide telestroke services [25]. In 2012, 97 potential telestroke networks were identified in the US alone, of which 56 were active [3].

IMPLEMENTATIONS

Technology Issues

In our own experience as well as from reports of other telestroke networks, there are a number of technical issues that have to be addressed during the implementation and maintenance of a telestroke network. First, the total cost of initiating a telestroke network is generally borne by the hub hospital. Spoke hospitals tend to participate in telestroke networks only if they are not required to pay for the technical equipment.

Soon after the implementation of telestroke networks, the equipment has to be maintained by the hub and spoke IT staff. The general lack of IT staff in spoke hospitals turned out to be a problem [26]. Currently, multiple vendors are available that offer professional and less maintenance-intensive systems. The vendors normally take care of the maintenance, repair and modernization of the telestroke equipment (e.g., Polycom, REACH Call, Tandberg, InTouch Health, and MEYTEC).

A further issue is the security of data. The information technology used must carefully control access to the telestroke data. In the US, data transfer must be compliant with Health Information Portability and Accountability Act requirements.

Modern telestroke equipment also integrates methods to store and communicate consultation findings. However, such a dedicated telemedicine software package is used in only 44% of US networks [3]. Alternatives include the use of paper, typing or the dictation of consultation findings [3].

Organizational Elements of Telestroke Networks

Before the implementation of a telestroke network, some important points must be considered. First of all, there have to be a need for the telestroke service, i.e., there must be small rural hospitals without a neurology department within the area surrounding the hub hospital. In the USA, most spokes are rural hospitals with fewer than 100 beds [3]. It should be further noted that the efficiency of a stroke network increases with the number of spokes [27, 28]. From a macroeconomic perspective, there should be at least 4 spokes associated with a telestroke network [27, 28]. In a survey from 2009, a mean number of spokes per hub of 7.6 was found, which was a significant increase over the mean for the years 2008 and 2007 [3]. The maximum number of spokes was found to be 28 [3].

The service offered and reimbursement model should also be further discussed. In particular, the service chosen might influence the possible reimbursement models. For example, two-way interactive video is often a requirement for any US federal and state reimbursement. A further question is whether brain imaging should be reviewed as part of the consultation process. However, the implementation of a new telestroke network that lacks the review of brain imaging or two-way interactive video can be seen as outdated, as in 2010,

94% of all (responding) US telestroke networks used both methods as part of their consultation process [3]. There are a multitude of vendors available that offer software and hardware for each aspect of telestroke service. Most telestroke implementations use the telestroke solutions of one vendor; ~20% of sites use a hybrid solution that combines the products of more than one vendor. The choice of one vendor for the whole telestroke solution is also highly recommended based on the experience of the authors, as technical incompatibilities and failures can represent a considerable drawback with respect to the confidence of the spoke hospitals in the telestroke network.

Additionally, some telestroke programs also offer a telephone-only consultation if needed. The composition and size of the telestroke team varies depending on the resources available at the hub. However, because 24/7 availability is highly needed, the personal and financial resources of the hub must be above a critical mass. This is especially true because most hubs provide neurology specialists from among their own personnel; only ~25% of the telestroke networks use outside-contracted specialists as part of the telestroke team. In all telestroke hubs, a physician is included. However, some programs also incorporate nurses or physician assistants [3].

The implementation of a telestroke network at the hub requires formal contracts or at least memoranda of understanding with spoke hospitals regarding consultation fees, equipment maintenance costs, data encryption and privacy.

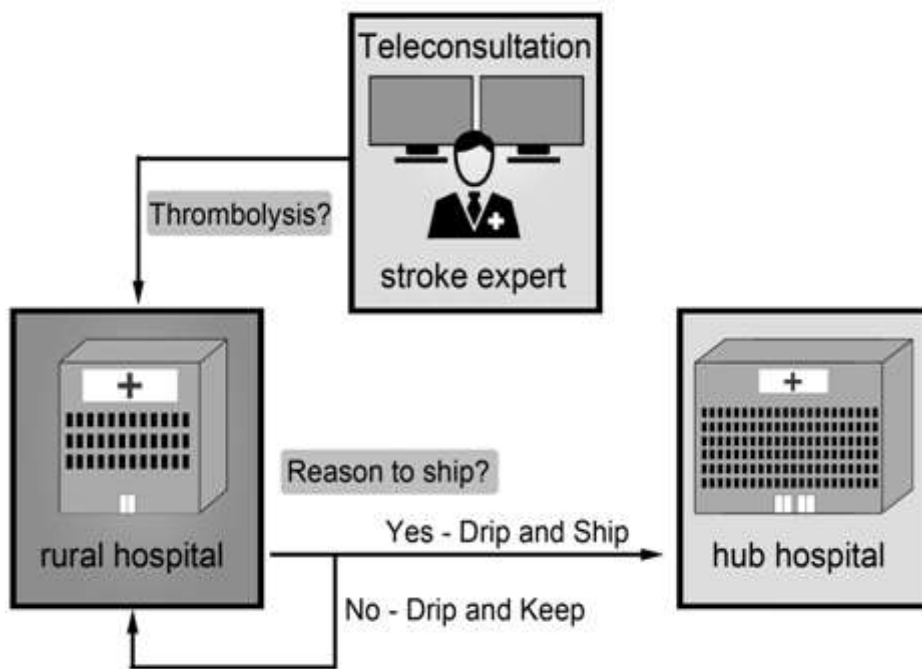


Figure 1. Telestroke in acute stroke treatment. The stroke expert has to decide whether the patient should be treated with intravenous rt-PA. The thrombolytic therapy is then given in the rural hospital. The stroke expert then has to decide whether there is a reason to ship the patient to the hub hospital. Such reasons might include, for example, the need for a neuroradiologic intervention in the case of a proximal vessel occlusion or increased intracranial pressure. If there are no reasons to ship, than the patient should be further treated in the rural hospital.

Telestroke-Associated Clinical Process of Care

There are multiple studies available reporting the successful performance of clinical processes and decision making for stroke thrombolysis in acute stroke by telemedicine [3, 4, 29-32]. The typical clinical process of care after patients arrival at a telestroke-associated emergency department is started after a stroke is suspected as a possible cause of symptoms. This initial step has to be performed by the physician at the local emergency department. In most telestroke networks, the clinical assessment of symptoms is quantified using the National Institutes of Health Stroke Scale (NIHSS). This score has shown high reliability and reproducibility in measuring neurological disability in stroke and has further shown high inter-rater reliability between a bedside examination and a remote examination using a telestroke network [21, 33, 34]. It was further demonstrated that assessment of the NIHSS by an experienced neurologist can be reproduced by non-neurologists and that the NIHSS is even robust when evaluated via prehospital assessment by a video cell phone [35-37]. The NIHSS score should be used to allow for comparisons with the current stroke studies that use this score.

After patient examination and NIHSS assessment, the evaluation of acute stroke brain imaging is the next fundamental step in telemedicine-based evaluation of the indication for stroke thrombolysis. Similar to the NIHSS score, teleradiological review of brain imaging also exhibits a high level of agreement. A recent study investigating the presence or absence of radiological contraindications to thrombolysis found an overall agreement of 91% between spoke radiologist, hub vascular neurologist and hub neuroradiologist [38]. A further study investigating the reliability of cerebral CT evaluation of early ischemic changes in 536 patients by stroke neurologists detected discrepant CT findings in 8%, while clinically relevant discrepancies were found in only 1.7% [39]. Accordingly, the American Stroke Association (ASA) guidelines recommend the use of teleradiology systems for timely review of brain CT and MRI scans in patients with a suspected stroke [40].

After evaluation of the patient and brain imaging, the hub hospital is notified and the on-call neurologist is connected. The patient is moved to a room containing the telestroke equipment while a nurse enters the patient's information into the system. The remote neurologist obtains the NIHSS score using real-time video assessment, views the brain imaging results and reviews the laboratory data if available. Thereafter, the remote neurologist makes his decision about the appropriateness of rt-PA administration and gives his recommendation to the local physician.

Prehospital Telestroke

Due to the small therapeutic window for rt-PA thrombolysis, strong efforts have been made to reduce the time between symptom onset and the start of the thrombolytic therapy.

Specific barriers were identified to the delivery of stroke thrombolysis, mainly at the hospital level, and removal of these barriers resulted in increased thrombolysis administration [41-43]. However, to minimize the time between onset of symptoms and treatment, the pre-hospital stroke management also has to be optimized [44]. The prehospital elements of the stroke treatment chain are more difficult for neurologists to influence because they are not under the control of stroke experts. Previous attempts to optimize the prehospital procedure

involved attempts to improve prehospital recognition of strokes by the use of stroke scales [45-47] and pre-notification of the emergency department about an impending stroke [48-51].

Beyond the use of stroke scales, it was hypothesized that diagnostic accuracy in prehospital settings could be improved if the examination was remotely performed by a hospital-based physician via video teleconferencing. Two studies of actors who simulated stroke symptoms in ambulances produced diverging results [52, 53]. While the first study found the evaluation of stroke neurological deficits to be valid and reliable and further found shortened treatment times [52], eight years later, the second study reported that prehospital telestroke examinations were not at a level acceptable for clinical use [53]. However, it is important to note that the negative result was mainly caused by problems in data transmission, which tends to be a problem for moving objects within current mobile communication technology networks. A further study has addressed this problem by bundling multiple 3G lines [54]. While it demonstrated the feasibility of prehospital teleconsultation, this study failed to demonstrate its superiority over regular EMS care [54].

Attempts to further optimize rt-PA treatment times have led to a system that provides a complete hyperacute stroke work-up preclinically by installing a point-of-care laboratory and a CT scanner into an ambulance (German Mobile Stroke Unit (MSU) and Stroke Emergency Mobile (STEMO)) [44, 55-57]. The CT images were transferred to the hospital for teleradiological evaluation [55, 58]. To provide the costly service of the STEMO in an efficient manner, it operates in a metropolitan area (Berlin) on patients who have been preselected using stroke identification algorithms during emergency calls [59-61]. In this setting, the STEMO has been demonstrated to decrease time to treatment without an increase in adverse events [44]. A particularly interesting point is that the STEMO ambulances are staffed by experienced neurologists trained in emergency medicine. Therefore, the video teleconferencing system available in the STEMO ambulances is not generally needed, but this capability might become important if ambulance-based thrombolytic therapy is performed without an on-board neurologist. However, the selection of the neurologists that staff the STEMO might introduce a slight bias, as one might speculate that the same neurologists might also exhibit above-average performance within a hospital. In our experience, the implemented stroke protocols are a major factor for the quality, speed and security of hyperacute stroke therapy, but these factors also depend on the experience and work load of the attending neurologist.

A similar Mobile Stroke Unit project was recently launched in the US [62]. During the 8 week run-in phase, ~2 patients per week were treated with rt-PA [62].

Telemedicine in Stroke Rehabilitation

Stroke is the most common cause of acquired disability. Stroke symptoms are dependent on the location of the brain damage and often affect motor function, speech, vision, cognition and sensation. It was estimated that 25% to 74% of stroke survivors require some degree of assistance or are fully dependent on caregivers for activities of daily living [63]. In particular, motor impairments were found to be the most critical factor influencing the patient's ability to live independently [64, 65]. While acute stroke treatment targets the prevention of further brain damage, stroke rehabilitation is able to achieve better recovery in terms of bodily

functions and activities of daily living to reduce disability and handicap during the following years [66].

As part of the post-acute stage, stroke rehabilitation is usually provided by healthcare professionals in a hospital or clinic setting and is often lengthy and resource intensive. Stroke telerehabilitation attempts to provide the same type of rehabilitation by an alternative method that does not require people to leave their homes. This constitutes the key advantage of telerehabilitation, which offers rehabilitation opportunities to people in remote areas that lack easy access to rehabilitation teams with expertise in stroke. It also reduces the burden of traveling long distances for people with severely restricted mobility. Additionally, telerehabilitation can be used to complement and enhance existing rehabilitation services. It might be possible to discharge patients from the clinical setting earlier and continue the rehabilitation process by telerehabilitation. In particular, long-term stroke care is considered underdeveloped, and many stroke survivors complain about a lack of intense, specialized rehabilitation after discharge from the rehabilitation clinic [67]. It is hypothesized that telerehabilitation might be a more convenient and less expensive means of rehabilitation after stroke. Despite the obvious advantages, there are considerable challenges associated with telerehabilitation [68]. The key issue is how to conduct assessments and treatments that need to be manually performed by the therapist? This shortcoming requires a modification of current techniques that do not need the “hands-on” support of the therapist [69]. A further question concerns the most appropriate form of communication between the rehabilitation professional and the patient [70].

There are multiple studies available that have investigated the effectiveness of stroke telerehabilitation and whether it improves outcomes. The mechanisms that lead to recovery are the same in conventional and telerehabilitation. Improvements in function during rehabilitation can be attributed to a combination of physiological recovery, neuroplasticity and compensation [71]. Therefore, the results of telerehabilitation can be directly compared with those of conventional rehabilitation strategies.

Most studies investigated the effects of telerehabilitation on motor function [72-77]. The training was performed at the patient’s home. These studies used customized computer-based training programs to improve the function of the upper [72, 75-77] or lower limbs [74]. A multitude of outcome measures were assessed, including physical function, independence in activities of daily living, quality of life and participant satisfaction. Some studies also assessed outcomes using a follow-up design [72, 73, 76]. All studies supplied the participants with the necessary video-conferencing hard- and software.

Most of the available studies compared telerehabilitation with an alternative intervention [75-77]. For example, Piron et al. used a virtual reality-based system to provide motor tasks to patients for 4 weeks [76]. This intervention was compared with traditional physical therapy. Both strategies were found to be effective, while the telemedicine intervention produced better outcomes in motor performance [76]. The same group investigated the satisfaction and improvements of patients undergoing a virtual reality therapy program at home compared with the same program in a hospital setting [77]. The authors reported significant improvements in motor function only in the telerehabilitation group and not in the group that trained in the hospital setting. Moreover, the group that received telerehabilitation showed a higher degree of satisfaction [77].

A further study compared the so-called Home Care Activity Desk training in a home setting with “usual care,” demonstrating no significant differences [75].

There are two studies available that compared two different telerehabilitation training strategies (repetitive tracking movements versus repetitive simple random movements) [72, 74]. One of these studies did not find a clear advantage of the tracking training over the same amount of practice of random movements after two weeks of training [72], while the other study found a significant advantage of the tracking training after 4 weeks of training [74].

A further study used a combination of technologies (video recordings, in-home messaging and phone calls) to compare functional mobility with “usual care” and found no significant differences between the groups [73].

Despite the availability of multiple investigations of the effectiveness of telerehabilitation, comprehensive studies that provide evidence of its effectiveness are still lacking [78, 79]. The reasons for this include the complexity and heterogeneity of stroke symptoms; the lack of robustness of the research methodologies used, which causes an insufficient ability to capture changes that are relevant to the treatment evaluated; and an often small sample size, which does not allow reliable data interpretation.

To date, there are also no studies available that have investigated the cost-effectiveness of telerehabilitation after stroke [79].

Motivations for Implementing Telestroke Programs

In the USA, only approximately 50% of patients have access to primary stroke centers within a drive time of 60 min, and half of all hospitals have no staff neurologists [3, 4]. A recent study estimated that the chance a patient will receive rt-PA is tenfold less in a rural emergency department compared to an urban primary stroke center [4].

Accordingly, the motivations to implement a stroke network are mainly to provide a community benefit, to improve clinical outcomes and process effectiveness and to provide knowledge [3]. A further driving force for hub hospitals to implement a telestroke network is that the hospitals likely already receive calls for help with patients, and it is generally very difficult to manage cases and indicate transfer to the hub without knowing whether there is indeed a neurological necessity.

Interestingly, revenue enhancements, cost reduction and bed management were among the lowest-ranking reasons to create or participate in a telestroke program [3]. However, these data were collected mainly from the physicians participating in the program, whereas the decision to create or participate in a program are made within the administrative hierarchy of a hospital, and such administrators did not participate in the cited survey [3]. Therefore, these results likely did not reveal the whole picture.

Barriers to the Initiation, Growth and Sustainability of Telestroke Programs

A survey of active telemedicine programs in the USA in 2009 identified 56 programs.

A survey of telestroke programs in the USA between 2000 and 2009 showed that the mean operating duration of the networks was 2.4 years [3]. The most important barriers to growth and sustainability were a lack of funds and a lack of reimbursement, respectively [3, 26, 80].

One possible solution is to increase the value associated with the treatment of acute ischemic stroke with thrombolysis (US: DRG 559). However, previous increases in this DRG were primarily due to the medication costs of rt-PA and not to possible increases in diagnostic and personnel efforts with these patients. In particular, the possibility of a telestroke consultation is not represented by this DRG. This is particularly illustrated by the fact that when a patient receives rt-PA in a spoke hospital and is then transferred to the hub, neither hospital receives this increased DRG [29, 80-82].

Also the inability to obtain physician licensure has been reported as an important barrier [3]. In particular, the lack of funds and a lack of technological support were the main barriers for spokes.

Malpractice protection is also an important barrier to the initiation and maintenance of telestroke programs. This, however, is a special problem in the USA. In most European countries, where the financial consequences of malpractice are more moderate, this did not constitute a relevant barrier.

CLINICAL EFFECTIVENESS

Participants

Pooled data analyses have clearly demonstrated that swift initiation of thrombolytic treatment with rt-PA is strongly beneficial to stroke patients [1, 2]. The therapeutic benefit of rt-PA is time dependent and is greatest when it is administered soon after stroke onset; its efficacy diminishes when it is administered at ≥ 4.5 hours post-onset [83-86]. The sensitivity of brain tissue to ischemia causes this time dependence of the effectiveness of rt-PA. Accordingly, the number needed to treat (NNT) for one patient to recover (mRS 0-1) increases with time (<1.5 h NNT=5; 1.5 h-3 h NNT=9; 3 h-4.5 h NNT=15) [86-88]. Adverse events are also time dependent; patients with earlier stroke onset-to-treatment times experience reduced symptomatic intracranial hemorrhage and a reduced likelihood of death [89]. Therefore, it is crucial to minimize the time between the onset of symptoms and treatment. For every 15-minute reduction of door-to-needle time, there is a 5% lower chance of in-hospital mortality [1, 2]. According to current guidelines, a door-to-needle time of 60 minutes is the official goal for treatment of ischemic stroke [40, 90]. In the US, the overall rate of stroke thrombolysis is <5%, and only 26.6% of patients who arrive in time to be treated with t-PA receive that medication within an hour after arrival, demonstrating the significance of in-hospital delays [2]. Some other hospitals have shown that improvements in the acute stroke management can reduce the door-to-needle time down to 20 minutes [43, 91]. There are also case reports that have demonstrated that under optimal circumstances, the door-to-needle time can be further reduced (down to 7 minutes) [92].

These data highlight that there is considerable room to optimize door-to-needle times. Factors that improve door-to-needle time include advance hospital notification by emergency medical services, fast access to a CCT, storage and administration of rt-PA in the emergency department, and full-time availability of an in-house stroke expert [93]. This requires the optimization of prehospital [44] and in-hospital stroke management as well as extensive

knowledge about the indications and contraindications for rt-PA. The optimization of stroke care is particularly difficult if few strokes occur and no in-house neurologists are available.

It is therefore probable that the addition of a consultation with a stroke expert via a telestroke network might accelerate the decision process and improve the quality of decisions. However, the costs and effort required to initiate and maintain a telestroke network have to be justified by evidence of benefit.

While most telestroke networks measure and evaluate the clinical quality of their programs [3], there are no randomized studies available that have investigated the influence of telestroke medicine on the functional outcomes of stroke patients. However, some non-randomized studies have investigated the clinical outcomes of patients [94-97].

One German study used a non-randomized design to compare 5 community hospitals that were included in a single telestroke network with 5 matched community hospitals that were not included in any telestroke network [94]. Within 2 years, 3122 patients were included. The main finding was a reduced rate ($p < 0.0001$) of patients with a poor outcome (44%) in spoke hospitals compared with the rate of 54% for patients treated in the control hospitals. A poor outcome was defined as an mRS > 3 , a Barthel index < 60 or institutional care or death after 3 months. The spoke hospitals demonstrated improved performance on a set of predefined measures of the quality of acute stroke care (e.g., diagnostic procedures, thrombolytic treatment (5% vs. 0%), physiotherapy, speech therapy). The length of stay in the hospital was significantly reduced in the spoke hospitals [94]. This study was complemented by a follow-up investigation of outcomes after 12 and 30 months, which showed that the patients treated in spoke hospitals exhibited significantly lower rates of death and dependency [96].

Another study compared the functional outcomes of patients treated with rt-PA between a spoke hospital and the hub and found no significant differences in functional outcomes after 3 and 6 months. The results were similar to those of the available randomized trials [98]. Similar results were obtained by a Finnish study [97], which also compared the functional outcomes (mRS) of rt-PA-treated stroke patients in spoke hospitals with the outcome of patients treated in the hub [97]. This study found a favorable outcome (mRS 0-2) in 49.1% (spoke) vs. 58.1% (hub) and symptomatic intracerebral bleeding in 6.7% (spoke) vs. 9.4% (hub). An interesting feature of this study was the high percentage of consultations that led to thrombolytic treatment (57.5%) [97]. Other studies have found rates of consultations that lead to thrombolytic treatment of 8.8% [94], 15.5% [23], 25% [99] and 29.8% [94]. The high rate of thrombolytic treatment compared to the number of consultations in the Finnish study indicates either a reluctant indication for a telemedicine consultation or a high degree of certainty regarding which patients are candidates for thrombolytic therapy and, in particular, which patients are not candidates. The same study also assessed changes in the use of thrombolysis in spoke hospitals compared to the time before their inclusion in the telestroke network. They found an increase in the use of rt-PA in the spoke hospitals by a factor of 2-3 [97]. However, this increase has to be interpreted cautiously because the time window for thrombolytic therapy was extended during the study from 3 to 4.5 hours [97].

Within the California-based STRoKE DOC trial (Stroke Team Remote Evaluation Using a Digital Observation Camera) the efficacy of stroke telemedicine decision making was prospectively assessed [99]. Within four spoke hospitals, 222 patients with a suspected stroke were randomly assigned to telemedicine or telephone consultation. This study found that telemedicine consultations resulted in significantly more accurate decision making (98% correct decisions) compared with telephone consultations (82% correct decisions). The 90

days functional outcomes (mRS) did not differ between the groups. No difference was found in the post-thrombolytic intracerebral hemorrhage rate. Also, mortality did not differ between the groups (with and without adjusting for baseline NIHSS score severity) [99]. The lack of differences in the functional outcomes is mainly due to the low number of included patients, such that the study was underpowered with respect to the expected effect. A further study also compared telemedicine with telephone consultations using a prospective randomized design [100]. This study found 89% correct treatment decisions if telephone consultations were used and 85% correct if telemedicine was used. No differences in functional outcomes were found. However, the cited study included only 54 patients (27 in each group), and technical problems were reported for 74% of the cases in the telemedicine arm [100], which strongly limits the interpretability of the results of this study [100].

Some other studies did not investigate functional outcomes but instead evaluated more specific parameters related to the telemedicine intervention. These studies are important to identify the characteristics of telestroke networks that are associated with good performance in specific aspects of stroke therapy. In a retrospective study, it was found that participation in a telestroke program increased the rate of thrombolysis administration to patients by 55% [101]. During one year of active telestroke work, the proportion of patients transferred to a primary stroke center after teleconsultation decreased from 44% to 19% [101], indicating a steep learning curve for the spoke hospital in handling acute stroke patients.

In conclusion, there is strong evidence that telestroke networks improve the functional outcomes of patients in rural locations. This is mainly achieved by increased rates of thrombolysis administration to patients with a decreased onset-to-treatment time.

COST-EFFECTIVENESS AND REIMBURSEMENT MODELS

Stroke is the leading cause of disability in developed countries, with costs of more than \$40 billion annually in the USA [26]. The financial systems underlying health care differ widely between countries. Most data are available from telestroke networks in the USA. The financial models for the reimbursement of telestroke networks might differ to some degree in different countries. In some countries, the telestroke service is paid for by the state health insurance (e.g., Finland). However, in most health care systems, telemedical consultation with a specialist results in no or minimal additional reimbursement by health insurances. Even in cases where an additional coverage is paid, these payments are described as inadequate [3]. Therefore, the reimbursement model requires the use of other sources to cover costs.

Macroeconomic Cost-Effectiveness of Telestroke Networks

With respect to the costs of initiating and maintaining a telestroke network, the question arises of whether these costs are worthwhile for the additional health benefits gained. The second important question is whether telestroke networks can deliver a positive financial outcome for the health system by improving outcomes and reducing long-term nursing expenses.

Analyses of the cost-effectiveness of telestroke networks are available only for a few countries. A US study developed a model to compare the costs and effectiveness of stroke care with and without a telestroke network over a 5-year time horizon [28]. The results suggested an increased rate of thrombolysis for patients treated within the telestroke network, leading to an increased rate of patients who were discharged home without requiring additional care. The cost analysis found a cost saving of ~\$100 000 for each spoke hospital, while the hub hospital had additional costs of ~\$400 000. Due to the size of the study network (seven spokes), this analysis resulted in cost savings overall. However, the authors also demonstrated the sensitivity of cost savings to the number of spokes within a telestroke network due to the fixed maintenance expenses of the hub [28].

A similar study was based on a Danish health economic model of thrombolysis treatment in a telestroke network [27]. The results suggested that the macroeconomic costs were balanced by savings in care and rehabilitation after two years and that the cost-effectiveness improved over longer time scales. However, this study also showed that the budgetary impact depends on the number of spokes and their capacity as well as on the organizational conditions at the spoke hospitals [27].

All of these studies of the cost-effectiveness of telestroke assumed that the financial effects are solely due to an increased use of rt-PA. Other potential causes for cost savings associated with telestroke consultations were not taken into account. A more general analysis was performed within the TEMPiS system. The costs of inpatient and outpatient care in a typical hospital population were investigated over 30 months. All of the patients suffered from a stroke but did not necessarily receive rt-PA. This study concluded that the additional costs of telestroke consultation were fully compensated by the cost savings in outpatient care [102].

Overall, the available studies that have analyzed the cost-effectiveness of telestroke concluded that from a long-term perspective, telestroke seems cost-effective compared with usual care, mostly because telestroke costs are upfront and the benefits of better stroke care are lifelong [27, 28, 30, 102-104].

Reimbursement Models of Hub Hospitals

Telestroke service incurs additional costs for the providing hospital. First of all, there are the initial equipment costs, which mainly depend on the level of service offered. During operation, personnel costs are an important component. Most hubs and spokes have access to dedicated technical support during the consultation process, which also contributes to the operating costs.

Reimbursement models vary widely between health care systems. In some countries like Finland, telestroke service is directly paid for by the state or health insurance companies. However, these cases are the minority. In most countries, it is the responsibility of the hub hospital as the service provider to reimburse the expenses. This is mainly done by on-call pay for telestroke consultations from the spoke hospital to the hub. Another model is the sharing of additional income for the spoke hospital that is caused by the telestroke network. This model is applicable in countries where the use of rt-PA and/or the initial attendance by a neurologist generates additional income for the spoke hospital. Some telestroke networks have formal agreements about the partition of this additional income. This model has the

advantage of a win-win situation for both hospitals. However, the downside of this reimbursement model is that telestroke consultations with diagnoses other than stroke will generate work instead of income for the hub hospital. In particular, in this model, the threshold for telestroke calls by spoke hospitals is very low, and the average reimbursement per call is low for the hub hospital.

Aside from direct reimbursements, a telestroke network binds the spoke hospitals more tightly to the hub hospitals. Patients who have to be shipped after a telemedicine consultation are mostly shipped to the hub hospital. The hub, however, can admit a patient only in case of free capacity. Eventually, this can lead to a decrease in free capacity and an increased degree of capacity utilization in the hub hospital, rendering the latter more cost efficient.

Reimbursement Models of Spoke Hospitals

In most cases, participation within a telestroke network does not cause expenses for the spoke hospital apart from the costs for technical equipment and telemedical maintenance costs. Depending on the contracts between hub and spoke hospital, telestroke consultations will be paid per call or alternatively, the additional income for the spoke hospital that is produced by the telestroke consultation is shared. In the second case, no reimbursement is necessary. In the first case, the additional income produced by the telestroke consultations has to compensate for the costs of the telestroke consultations. Some insurance companies in some US states reimburse spoke hospitals for telestroke consultations, but these payments were described as insufficient [3]. To compensate for the increased diagnostic and therapeutic effort, Medicare has created a higher-value diagnostic related group (DRG-559) for the use of rt-PA in acute stroke. However, the increased reimbursement is only paid if the patient remains in the hospital where the rt-PA was administered. In the case of a “drip and ship” case, neither the spoke nor the hub hospital receives that increased reimbursement. This system is particularly questionable in light of the recently published results from intervention studies, which demonstrated improved functional outcomes after rapid endovascular treatment for patients with an acute ischemic stroke with a proximal vessel occlusion [8-11].

As another example, in Germany, thrombolytic therapy produces additional income, similar to the DRG model in the US. Furthermore, German insurance companies pay additional reimbursements if the patient is treated within a “stroke unit” and is examined by a neurologist. An initial telestroke consultation is accepted by the insurance agencies similarly to neurological bedside examination.

Additionally, telestroke consultations enable the spoke hospital to deliver rt-PA onsite instead of transferring the patient to a specialized stroke center [40, 105, 106]. This motivates many rural hospitals to implement their own system of stroke care, moving from a “ship and drip” to a “drip and keep” model. This has led to a retention rate of 60-70% of patients at spoke hospitals, with significant cost savings [32, 103].

PROBLEMS AND LIMITATIONS IN TELESTROKE

Quality of Service

It has been demonstrated that the percentage of consultations that lead to rt-PA use vary widely [23, 94, 97, 99]. Also, within a single telestroke network, there are variable results on the percentage of rt-PA delivery in spoke hospitals [62]. The reason seems to be multifactorial. A recent study identified the presence of a stroke nurse coordinator at the spoke as an important factor for improved treatment of ischemic stroke with rt-PA [62]. However, it remains an open question whether this is solely a direct effect of the stroke nurse coordinator or whether the nurse coordinator is an expression of the commitment for acute stroke treatment that a spoke hospital is willing to do aiming to increase the quality of the telestroke network. This commitment could also add to this effect through a multifactorial influence.

Technical Problems

When telestroke networks were first being put into use, the lack of bandwidth caused often an insufficient video quality. Today, most video-conferencing systems offer high quality videos. However, depending on the organizational structure of the telestroke network, the stroke specialist is not always at the hub hospital and may have to perform his telestroke consultation from home. There can be a lack of bandwidth. However, in our experience, even if the video quality is reduced, this does not usually affect the ability to obtain an accurate NIHSS score.

Interestingly, a prospective study from 2010 found technical problems during the performance of telestroke in 74% of consultations [100]; these problems affected video, audio, the internet connection, image forwarding or multiples of these [100].

Non-Stroke-Telemedicine

Imagine the following situation: it is night at small rural hospital, and there is only one physician in his second year at the emergency department as a patient is brought by the paramedics with unclear neurological symptoms that cannot be interpreted as stroke. The physician knows that there is a very experienced neurologist only one call away who can examine the patient through the telestroke equipment. Why should he not call? In particular, if the contract with the hub does not involve a pay-by-call model and instead employs a profit-sharing model, there is an increased tendency to use the telestroke network for these cases as well. Indeed, it would be morally unacceptable to withhold this expertise from the patient. In our own experience, there is a massive trend toward using the telestroke network not only for stroke patients, but also for patients with stroke mimics and other neurological syndromes, and ideally, this should be considered a feature instead of a bug.

US-Specific Problems – Licensing and Accreditation

In the USA, the telestroke consultant must be licensed by the state and hold credentials at the local hospital. Today, there are no national or multistate licenses for telestroke that could reduce the burden on the stroke consultant to be licensed in multiple individual states if the telestroke network extends across state borders [107]. In Europe, there is no such thing as “state licensing” within countries. The telestroke consultants have to be licensed in every country. Currently, there is no Europe-wide licensing. However, this does not constitute a problem in this context due to the lack of telestroke networks across country borders.

PERSPECTIVES

In the last decade, telestroke has undergone rapid growth and is now advancing into the mainstream of acute stroke care. Telestroke has been shown to improve the functional outcomes of patients and is also cost-effective. However, telestroke networks are still locally organized, and there is mostly a lack of national and international standards for the technology, quality of care and organizational structure of telestroke networks. Today, the technological advancements of broadband networks enable even the smallest and most remote hospitals access to state-of-the art neurological stroke expertise. This opportunity is only limited by the will of participants and in some countries (particularly the USA), national and state regulations. Ultimately, telestroke will diminish geographical disparities in access to expert care, with the aim of ensuring that all patients with acute stroke receive optimal care. This aim also requires the political will to ensure that no hospital should treat acute stroke patients without neurological expertise. In this context, changes to reimbursement are also required that will provide incentives for stroke specialists to provide on-call cover.

Discarding the “Ship and Drip” Model

There should be general agreement that the “ship and drip” model is not state-of-the-art treatment for acute stroke patients. A hospital that is using this method should be integrated into an existing telestroke network. Every effort should be made to ensure that thrombolytic treatment can be provided by every hospital that sees patients with symptoms of an acute stroke. A “drip and ship” model could then be further extended to a “ship and keep” model where unproblematic stroke patients are treated, and the telestroke network can then be used for further directions.

Non-Stroke-Telemedicine

As stated above, the use of telestroke networks to evaluate non-stroke neurological cases should not be seen as misuse but as an important extension to the ability to bring expert neurological knowledge to rural locations. A recent retrospective study evaluated 352 teleconsultations with a non-stroke diagnosis [108]. It was found that the time required for a

teleconsultation for a non-stroke patient nearly doubled the time of stroke cases (26.5 minutes vs. 14.3 minutes). It was further found that diagnostic accuracy was not as exact in non-stroke cases compared to acute stroke cases [108]. However, from our own experience, it should be noted that a stroke is in most cases, one of the most recognizable neurological diseases. Furthermore, telestroke equipment was developed to evaluate acute stroke patients. Therefore, it is less feasible, however possible to use this equipment to investigate impaired oculomotor features or movement disorder symptoms, for example.

The E-Stroke Unit

Patients with acute stroke not only require specialized neurological attention during the hyperacute stage but also benefit from organized stroke care (“stroke unit”) during the following days [109, 110]. Most of the requirements of such a stroke unit can be provided by smaller hospitals (e.g., cardiopulmonary monitoring, speech and language therapy, and physiotherapy). However, specialist coverage of these patients by neurologists often remains the main challenge. Today, the question is whether repeated teleconsultations can compensate for the lack of an in-house stroke expert. Moreover, the idea of mobile telemedicine workstations has been discussed to enable the remote stroke expert to become virtually present at the patient’s bed to supervise the stroke care [34].

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Chapter 32

IMAGING IN ACUTE STROKE

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ABSTRACT

Acute ischaemic stroke is one of the neurological urgencies. Intravenous thrombolytic therapy should be initiated within 4.5 hours from symptoms onset; unfortunately, many patients with acute stroke are admitted to hospital with an acute stroke with unknown time of onset or after the therapeutic window.

Nonenhanced CT is currently used to exclude contraindications to fibrinolytic therapy, and CT Angiography can show the presence of a thrombus within a large vessel. On the other hand, advanced imaging modalities, such as CT and MR perfusion, seem to be new promising tools in the pre-treatment assessment of patients as a surrogate of ischaemic penumbra. The research of radiological markers of ischaemic but savable tissue might change some criteria for patient selection for treatment. However, clinical trials performed over recent years have often shown conflicting results. This inconsistency was probably partially caused by different criteria adopted for patient selection, and by the use of different imaging modalities and technical parameters. Further randomized controlled studies are essential in order (i) to establish if perfusion-based criteria to select patients for treatment lead to a better outcome within and/or after 6 hours from symptoms onset; and (ii) to investigate if there are reliable radiological criteria to exclude patients that do not benefit from reperfusion.

Keywords: acute ischaemic stroke, CT, CT perfusion, DWI, MR perfusion, perfusion-diffusion mismatch, FLAIR

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INTRODUCTION

Acute ischaemic stroke can be treated if therapy is administered in the early phase of the disease. Intravenous thrombolysis with recombinant tissue plasminogen activator (rtPA) is the first-line therapy and should be initiated within 4.5 h from symptoms onset in order to obtain a good clinical outcome [1]. In selected patients, endovascular treatment represents an integrative option and allows a wider therapeutic time window (usually 6 hours in the anterior circulation and even more in the posterior circulation).

The treatment of ischaemic stroke is based on brain imaging to guarantee appropriate patient selection and to avoid complications [2]. The goals of neuroimaging are: (i) to exclude contraindications to reperfusion; (ii) to rule out differential diagnoses; (iii) to detect arterial occlusions; (iv) to evaluate the infarction core and ischaemic penumbra. Therefore, the role of imaging can be summarized in the “four P’s” [3] that include the assessment of Parenchyma, Pipes, Perfusion and Penumbra. Different imaging techniques are specifically adopted to achieve each aim: CT scan without contrast administration (NCCT) and conventional MRI with DWI are used to study the brain parenchyma; CTA or MRA are useful for vessel assessment; and CT Perfusion (CTP) or MR-PWI allow the evaluation of perfusion and the penumbra.

NONCONTRAST COMPUTED TOMOGRAPHY (NCCT)

NCCT is the mainstay of the acute stroke workflow based on its high level of evidence in the exclusion of intracranial haemorrhage and in the revealing of stroke mimics (Figure 1a-b) [4]. It has several advantages: it is fast, widely available and feasible.

A radiological contraindication to fibrinolytic therapy is the presence of large hypodense acute ischaemic changes in more than one-third of the middle cerebral artery (MCA) territory [5]. A quantitative method useful to select patients who are not suitable for revascularization is the Alberta Stroke Program Early CT Score (ASPECTS) [6]. According to this method, the MCA territory in each hemisphere is divided into 10 regions with an overall maximum score of 10 points. Then, for each region in which acute ischaemic CT signs are recognised, one point is subtracted from the maximum score. Patients with an ASPECTS below 7 are less likely to benefit from thrombolysis.

NCCT also provides early signs of ischaemic stroke but with limited sensitivity (31% at 3 hours and 82% at 6 hours) [7] and reliability [8], especially in the posterior fossa and for lacunar infarctions [9]. Furthermore, early signs of ischaemic stroke are subtle on NCCT and have a high intra- and inter-observer variability, but they are not a contraindication to fibrinolytic therapy [4]. They include the loss of gray-white junction differentiation, the sulcal effacement and the loss of delineation of the basal ganglia and the insular ribbon (Figure 1c). The hyperdense artery sign (or dot sign) is attributed to the presence of a thrombus within the lumen of a large vessel and it is detected in about 30% of thrombosed intracranial vessels [10].

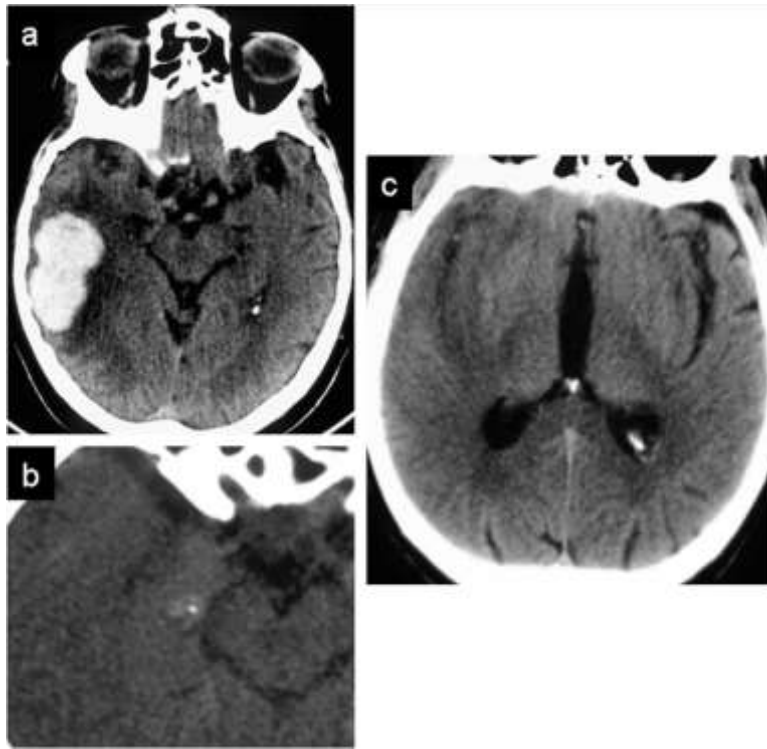


Figure 1. NCCT allows the immediate detection of the hyperdensity of blood in haemorrhagic stroke (a) and reveals several stroke mimic conditions, e.g., a Todd's palsy after an epileptic seizure secondary to a small cavernous angioma of the right hippocampal formation (b). In (c) early signs of ischaemic stroke are shown: note the Sylvian fissure effacement and the loss of delineation of the insular ribbon in the right hemisphere.

MAGNETIC RESONANCE IMAGING (MRI)

There is evidence that MRI, as well as NCCT, can be used to rule out intracranial haemorrhages [11] and stroke mimics in patients that are candidates for fibrinolysis [4]. Furthermore, MRI has a higher sensitivity than NCCT in detecting ischaemic tissues and avoids exposure to ionizing radiation. Nevertheless, several constraints limit its use in patients with ischaemic stroke. The first limitation is the lack of availability of the MRI service on a 24/7 basis. Moreover, the management of the acute phase of stroke, respecting the MR compatibility, is often difficult and time consuming. A further limitation is related to the MR protocol for patients with stroke that has to be essential and rapid to compete with the CT-based protocol.

The most important MR sequence is Diffusion-weighted imaging (DWI) that detects areas of restricted water diffusion in the brain as a sign of cytotoxic oedema. The performance of DWI for this purpose is excellent, having high sensitivity (88-100%) and negative predictive value (96%) just a few minutes after the stroke onset [12], consistently outperforming conventional MR sequences and CT (Figure 2) [13]. In patients with stroke the area of restricted diffusion is considered as the ischaemic core [14], but reversible DWI lesions after stroke are reported, often with an embolic origin [15].

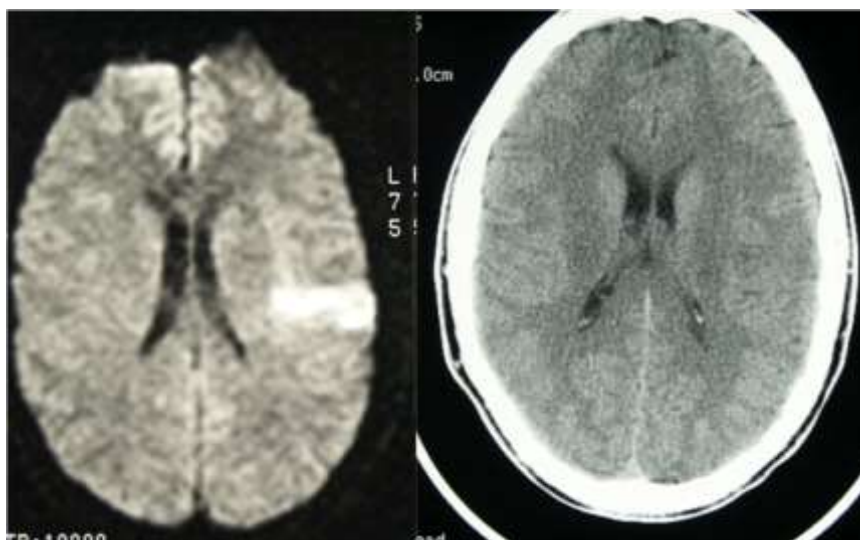


Figure 2. DWI is the most powerful method to reveal cerebral ischaemia. In the left of the image, DWI reveals an area of signal hyperintensity in the left frontal lobe corresponding to the cytotoxic oedema during the hyperacute phase of stroke, when CT scan (in the right side of the image) is still unremarkable.

Similar to NCCT, DWI is used to exclude patients at risk for haemorrhagic complications during fibrinolysis. Exclusion criteria are: (i) a large infarct with cytotoxic oedema that involves more than one-third of the MCA territory; (ii) an infarct volume larger than 70 ml; and (iii) an ASPECTS < 8.

The MR protocol in acute ischaemic stroke has to be implemented with MR techniques for vessel imaging (MRA) such as Time of flight (TOF). MRA is a flow-based technique and does not require injection of contrast medium, but it is prone to specific artefacts that tend to overestimate the stenosis. With respect to CT Angiography and mainly to DS Angiography, MR Angiography is able to detect proximal vessel occlusions but it is not sensitive in identifying occlusions of distal branches.

Fluid attenuated inversion recovery (FLAIR) sequences are usually included in the MR protocol for the patient with acute stroke. Signal hyperintensity in FLAIR images is detectable later than reduced diffusion in DWI [16]. In patients with undated or wake-up stroke, FLAIR images can be used to estimate the time from the ischaemia onset. Ischaemic lesions that are detectable in DWI but not in FLAIR images are probably dated within the therapeutic window of 4.5 hours and can be safely treated with reperfusion therapy [17]. Furthermore, patients with stroke of unknown time of onset and selected by means of DWI/FLAIR mismatch have a good clinical outcome after recanalization therapy [18].

In dedicated centres, the duration of MRI exams to assess patients with acute stroke is less than 15 minutes. Although this scan time does not significantly prolong the time to treatment, the use of CT is more appropriate and recommended for good clinical practice [19].

CT ANGIOGRAPHY (CTA)

CTA with multi-detector CT technology is an alternative to catheter angiography in the evaluation of intracranial and extracranial vasculature, providing information about the location and extent of the thrombus (Figure 3). CTA revealed higher sensitivity than MRA for both intracranial stenosis (98% versus 70%, $P < .001$) and occlusion (100% versus 87%, $P = .02$). Moreover, CTA had higher positive predictive value than MRA for both stenosis (93% versus 65%, $P < .001$) and occlusion (100% versus 59%, $P < .001$).

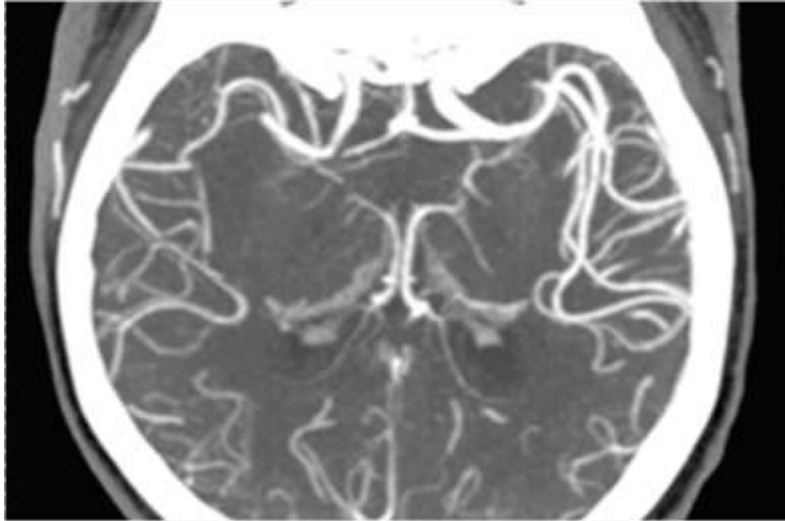


Figure 3. CTA of intracranial vessels reveals the occlusion of the upper branch of dichotomization of the right middle cerebral artery.

CTA is performed by means of an intravenous injection of iodinated contrast media; the scanning is timed with the injection and starts from the aortic arch up to the level of intracranial vasculature. CTA provides important information for the planning of the endovascular approach to thrombectomy due to its panoramic view, the ability to always depict the presence or absence of the intraluminal thrombus (the target of endovascular therapy) [21], and because it reveals anatomical variations or kinking of the vessels.

CTA gives no information about the dynamics of blood flow but it can provide an estimation of the collateral circles from leptomeningeal arteries. Collaterals play an important role in ischaemia because they increase the reperfusion rates and decrease both the infarct core size and the hemorrhagic conversion, thus improving clinical outcome with recanalization therapies [22]. An ischaemic area surrounded by poor collateral circles has a low probability of rescue. Collateral circles in the ischaemic hemisphere can be estimated by comparing the backfilling of the pial arteries beyond the blocked artery to the circle of the unaffected hemisphere [23]. The major drawback of CTA is that it is monophasic; for this reason, it is possible to mistime the contrast filling of blood vessels and this can cause an overestimation of the ischaemia. In order to avoid this disadvantage, it is possible to perform multiphase CTA. This is a relatively recent technique that obtains a series of time-resolved angiograms by injecting a single dose of iodinate contrast media. After the arterial acquisition

of the vasculature from skull base to vertex timed with a bolus trigger, a further three acquisitions are scheduled to obtain a late arterial, venous and late venous phases [24].

Until the recent review of the AHA guidelines for stroke management, a vascular study was recommended in patients with acute stroke after the therapeutic window for rtPA administration, especially if either intra-arterial thrombolysis or mechanical thrombectomy was contemplated for management [4]. With the affirmation of the endovascular thrombectomy, CTA became the second recommended imaging technique after NCCT, when selecting patients for endovascular therapy [25]. In fact, the new revised guidelines for stroke management state with high level of evidence that non-invasive intracranial imaging should be obtained as quickly as possible in patients with stroke [26].

MR PERFUSION

Perfusion techniques are advanced MR methods introduced to explore the physiopathology of stroke and to improve patient selection for treatment, in order to achieve a better outcome of treated patients and to reduce futile treatments.

Some different definitions of ischaemic penumbra have been proposed over the years. Basically, the penumbra is an ischaemic and functionally impaired brain tissue whose haematic flow is lower than the threshold of electrical failure and higher than the threshold of energy and ion pump failure [27]. The impaired tissue is still viable and might be saved if reperfused before it is irreversibly damaged and recruited into the infarct core [28]. The ischaemic penumbra is becoming a possible milestone in the workflow of patients with acute ischaemic stroke. All medical and interventional treatments aim to save as much brain tissue as possible and to obtain as better clinical outcome as possible. However, not all impaired tissue can be saved: in fact, unlike the ischaemic core, the functionality of ischaemic penumbra can be theoretically restored by means of vessel recanalization and tissue reperfusion. If this is true, the absence of ischaemic penumbra also immediately after symptom onset would make any therapy useless. On the contrary, the presence of ischaemic penumbra even beyond the accepted time window for reperfusion might be a criterion to select patients that could benefit from treatment also at later stages.

Unfortunately, this is not so easy to achieve in daily practice. The first problem concerns the definition of ischaemic penumbra: it is not a radiological definition but there is a need to discover its radiological counterpart. Description of penumbral tissue using imaging used to come from PET studies, whereas nowadays it is usually evaluated by using MRI and is supposed to be represented by the perfusion-diffusion mismatch, that is the difference between the volume of perfusion and diffusion impaired tissue. There is currently no agreement about the most reliable perfusion parameters or the best cut-off for the evaluation of the penumbral volume and the differentiation between ischaemic penumbra and benign oligoemia. As a consequence, different trials use different landmarks making comparison among studies hard.

Treatment of patients with acute ischaemic stroke aims to arrest the evolution of penumbral tissue into infarction, limiting the final infarct volume. Which are the patients that could benefit from the treatment and what are the best criteria for their enrolment? Is there a time limit after the symptoms onset for its safety and effectiveness? The possibility of using

radiological rather than temporal criteria to select patients for reperfusion might also allow to treat patients that today are excluded because of undated acute stroke.

In clinical practice and mainly in patients with acute stroke, cerebral perfusion can be measured adopting an MR technique that uses a non-diffusible exogenous tracer, named dynamic susceptibility contrast MR imaging (DSC-MRI). DSC-MRI is based on the local magnetic field inhomogeneities induced by the first passage of a contrast bolus of paramagnetic tracer through the cerebral capillary network. Echo-planar sequences allow the detection of rapid signal changes in the brain parenchyma with an adequate temporal resolution. Signal-time course data can be converted to relative tracer tissue concentration-time course data, and haemodynamic parameters such as cerebral blood volume, cerebral blood flow and mean transit time can be derived. Cerebral blood volume (CBV) refers to the blood volume fraction of the tissue and it is expressed in ml/100 g of tissue. Cerebral blood flow (CBF) is a measure of the blood volume delivered to the tissue in a certain time and it is expressed in ml/100 g of tissue per second. Mean transit time (MTT) is the time that a volume of tracer takes to traverse a specific volume of tissue and it is expressed in seconds. Absolute quantitation of cerebral blood flow has been attempted using DSC-MRI tracer kinetics analysis that measure arterial input to the brain. The arterial input function (AIF) is usually generated by manually placing a ROI at the level of the middle cerebral artery.

CBV maps are obtained by fitting the first pass response in the concentration time curves to a gamma variate and then integrating the fitted curve.

$$CBV = \frac{k_h}{\rho} \frac{\int C_{voi}(t)dt}{\int C_a(t)dt}$$

CBF calculation requires the deconvolution of a residual function from an arterial and tissue concentration-time data.

$$C_{voi}(t) = CBF \ C_{art}(t) \overset{\Delta}{\underset{\Delta}{\Delta}} R(t)$$

MTT maps are calculated based on the central volume principle, from the ratio of CBV to CBF.

$$MTT = \frac{CBV}{CBF}$$

Although the absolute perfusion measurement has been demonstrated to be in good agreement with accepted physiological flow rates, there are intrinsic difficulties in the quantitation because of the AIF measurement, bolus delay and dispersion.

Parameters directly derived from the tissue concentration-time curves are: (i) maximum peak concentration (MPC), that is the highest value of the tracer concentration after injection; (ii) area under peak (AUP), that is the intergral of the tissue concentration time curve; (iii) time to peak (TTP), that is the time from the tracer injection to the maximum concentration obtained; (iv) time to arrival (BAT), that is the time from the tracer injection to its arrival to

the tissue; and (v) full width at half maximum (FWHM), that is the width of the concentration-time curve at half of its maximum amplitude. These haemodynamic parameters are useful to obtain qualitative information in several pathologies but depend on many variables such as total body vascular volume, cardiac output, injection rate, injection dose, cannula size; therefore, they can not be directly compared either between different subjects or in the same subject in different times. Nonetheless, the reduction of their variability can be achieved using an internal standard of reference, such as the normal grey or white matter, and computing semi-quantitative or relative values.

With the affirmation of thrombolytic therapies, the need of a method to select patients that may benefit from systemic or intravascular treatment became a matter of urgency. Besides the area of reduced diffusion that identifies the infarct core, different PWI methods have been described to define the penumbra. The most employed method is the mismatch between altered DWI and PWI areas, but also thresholds of TTP and MTT are used to define the area with reduced perfusion (Figure 4). In the DEFUSE and EPITHET trials, the mismatch is defined as a 20% difference between PWI and DWI areas [29]. Furthermore, although DWI-PWI mismatch allows the identification of the brain tissue at risk of infarction, an ideal perfusion using non-time dependent parameters is not yet available.

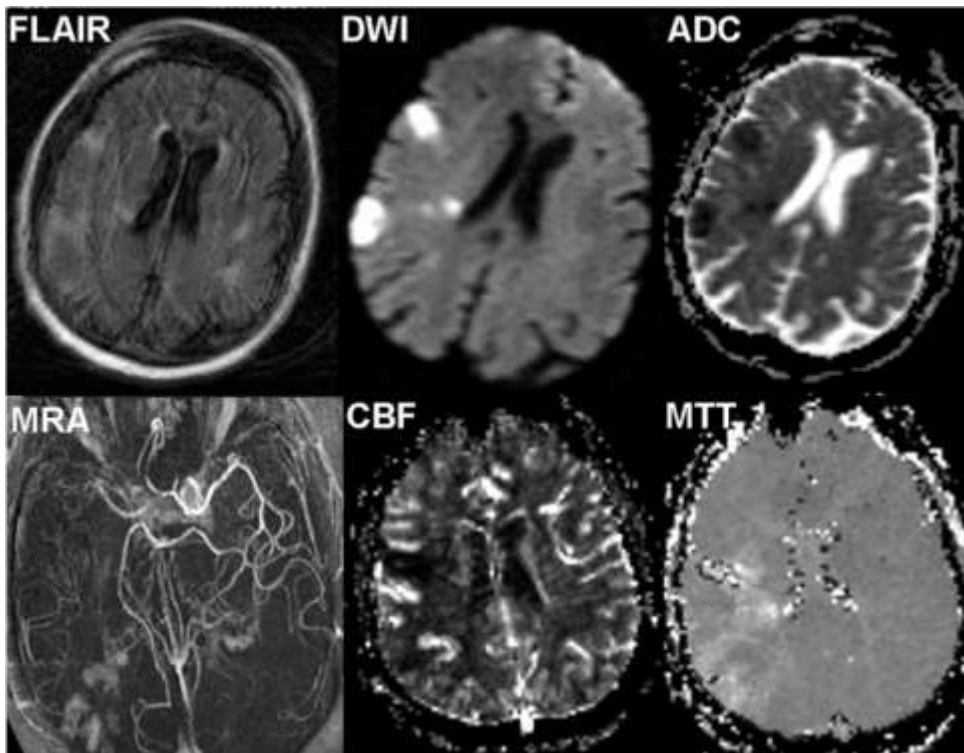


Figure 4. MR evaluation of acute stroke. In conventional FLAIR image there are multiple areas of signal hyperintensity involving the cortical ribbon in the right frontal lobe, characterized by signal hyperintensity in DWI and reduced water diffusion in ADC map. These areas of cytotoxic oedema are considered to correspond to the irreversibly damaged tissue (infarct core) caused by the occlusion of the right middle cerebral artery visible at MRA. MR perfusion obtained with DSC techniques allows calculating CBF and MTT maps that reveal a large area of hypoperfusion extending beyond the infarct core. The mismatch between DWI and PWI areas is the ischaemic penumbra.

The potential usefulness of perfusion imaging in acute stroke includes: (i) the identification of the ischaemic core at increased risk of hemorrhage after thrombolysis; (ii) the identification of salvageable brain area with thrombolysis performed beyond the standard therapeutic window; and (iii) the management of patients with unknown stroke onset (i.e., wake-up stroke) [30].

Although widely available and used, the diagnostic and clinical usefulness of perfusion techniques have not been proven in controlled studies of adequate power [4].

CT PERFUSION (CTP)

CTP provides the detection of the ischaemic penumbra and the infarct core. Multidetector CT scanners, tracking the iodinated contrast media as an exogenous tracer through capillaries into veins, are able to display, for each pixel, a curve representing the contrast media concentration over time. Deconvolution analysis of arterial and venous concentration-time curves provides quantitation of CBV, CBF and MTT. [31] CBV is calculated from the area under the time-density curve; CBF is derived from the gradient of the wash-in of the time-density curve; MTT is derived from the central volume theorem according to the equation $MTT = CBV / CBF$ [32].

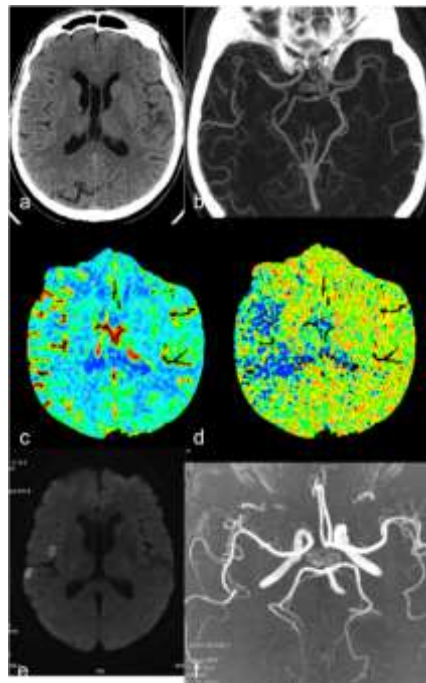


Figure 5. CTP evaluation of penumbra. Patient with acute onset of left hemiplegia and unremarkable NCCT (a). CTA (b) reveals thrombotic obstruction of the bifurcation of the right middle cerebral artery with good collaterals for the backfilling of distal vessels. CTP maps display a normal or slightly increased CBV (c) that corresponds to an increased MTT (d) in the middle cerebral artery territory and indicates penumbra with salvageable tissue if re-perfused. No clear ischemic core is identifiable. After recanalization, DWI (e) demonstrates only small foci of cytotoxic oedema within preserved middle cerebral artery territory. MRA (f) confirms the complete recanalization of the intracranial vessels.

CTP exploits the CBF-CBV mismatch to obtain the ratio between the infarct core and the penumbra [33]. Areas with increased values of CBV and MTT (or reduced CBF value) represent hypoperfused but viable tissue at risk of infarction (ischaemic penumbra), whereas areas with decreased CBV (or CBF) and prolonged MTT values represent irreversibly damaged tissue (infarct core) (Figure 5) [34]. If some studies state that the mismatch between CBV and MTT (or CBF) maps roughly represents the penumbra, other studies suggest the use of quantitative measurements of CBF as a predictor of the infarct core. A CBF reduction to 30%-50% with respect to the CBF of the contralateral hemisphere is considered a sign of irreversible tissue damage [35].

Further semi-quantitative parameters can be obtained from the analysis of the density curves, such as the TTP curve. By showing the time to reach the apex of the time-density curve, the TTP curve reflects the time that the contrast bolus takes to reach the tissue and, when increased, it is considered a sensitive marker of cerebral ischaemia.

Compared to MR perfusion techniques, CTP has a limited spatial coverage (but can be alleviated by the use of multi-detector CT scanners with more than 128 detectors) and is less sensitive in the detection of small lacunar lesions [36]. Nevertheless, CTP is more available, easy to manage in an acute setting, and together with NCCT and CTA, forms a continuum of diagnostic tools.

ROLE OF ADVANCED IMAGING IN STROKE: LIMITATIONS

A fast and accurate imaging to define perfusion and penumbra is desirable, and the capability to detect the potentially salvageable brain tissue in a single subject would be preferable to the mere exclusion of contra-indications provided by standard imaging. However, clinical trials which tested advanced imaging techniques for patient selection produced conflicting and inconclusive results [37-40]. In general, patients with perfusion mismatch treated with rtPA within 6 hours from symptoms onset had significantly attenuated infarct growth and higher reperfusion rate; however, mortality and clinical outcomes were not different compared to patients without mismatch [41]. A favourable penumbral pattern on neuroimaging does not identify patients who would benefit from endovascular therapy for acute ischaemic stroke if onset to treatment time has been too long [42].

To date, delayed thrombolysis in mismatch patients has not been confirmed to improve clinical outcome and, therefore, delayed treatment according to mismatch selection cannot be recommended. This conclusion is related to the definition of imaging-based mismatch which is still evolving [43] and is influenced by the physiopathology of mismatch and also by technical limitations. From a conceptual point of view, any attempt to determine the status of ischaemic and oligemic tissue with imaging is a snapshot in time [44] whereas the penumbra is a developing phenomenon and any perfusion threshold level is time dependent [45]. For this reason, a tissue is viable and salvageable at a certain threshold in a moment but it is no longer salvageable at the same threshold at a later time.

Beside these conceptual limitations, some technical constraints often make the results of different trials equivocal and the comparison among them hard. An attempt to clarify the terminology is on-going in the scientific community, aiming to reach common methods of acquisition and analysis of perfusion [46].

The parameters used in CTP and MR-PWI to describe the ischaemic core and the penumbra are different and do not always overlap [47]. In MR-PWI, the use of DWI for the identification of the ischaemic core has an important limitation: not all DWI lesions are irreversible [48] and they can disappear in a not negligible number of cases (24%) [49]. In both MR-PWI and CTP, different perfusion parameters (CBF, CBV, time to maximum, MTT, TTP) are combined to define ischaemic core and penumbra in ways which often differ among trials [50].

The most robust recognized method to estimate perfusion parameters is the deconvolution method [51, 52], even though assumptions underlying deconvolution algorithms have been questioned. In fact, it is stated that the proportion between signal change and tracer concentration in dynamic susceptibility contrast perfusion is maintained only for specific conditions [53]; moreover, the method adopted in clinical practice for the AIF selection influences blood flow measurements [54]. In Figure 6 we report an example of two different CBF maps depending on the method used to select the AIF (manually drawn with a ROI-based method proximally to the middle cerebral artery or automated selected using a home-made algorithm) [55]. Furthermore, perfusion parameters are affected by bolus delay and/or dispersion [56], which are related to the tracer-bolus geometry and concomitant neck vessel pathology. Automated methods to correct the bolus delay and the dispersion are described [57], but the use of these algorithms in software for clinical practice has not yet been implemented. Quantitative evaluations of perfusion parameters are also prone to error depending on different deconvolution algorithms [58] and software adopted for the analysis [59]. Therefore, the most reliable estimation of the size of the final infarct tissue can be assessed by computing CBF and MTT maps by means of a single value decomposition algorithm and a delay-insensitive software.

In order to improve the standardization and reproducibility of advanced imaging interpretation and to extend the use of this new technique also in clinical practice (rather than only in highly specialized academic centres), an automated, rapid and robust CTP-based mismatch selection method (RAPID) [60] has been developed and adopted for imaging-based selection in the EXTENDED IA trial [61].

The lack of standardized perfusion thresholds used to define penumbra and ischaemic core make the comparison among trials difficult. The use of several perfusion analysis methods and the multiple definitions of tissue at risk both help to explain the myriad of perfusion thresholds reported in the literature [62]. Recently, alternative perfusion methods encompassing specific thresholds to make predictions have been developed. These models use FLAIR, PWI or DWI images for the analysis of voxel intensity at onset with respect to the observed tissue fate, thus estimating the viable tissue at risk [63]. The voxel-wise analysis may use spatial clustering to increase the likelihood of voxels to be identified with penumbra or benign oligoemia. A further method to predict the tissue fate is to display the spatial gradient of a parameter instead of the parameter itself. Gradient of CBV seems to be able to distinguish between benign hyperemia and penumbra surrounding the ischaemic core [64].

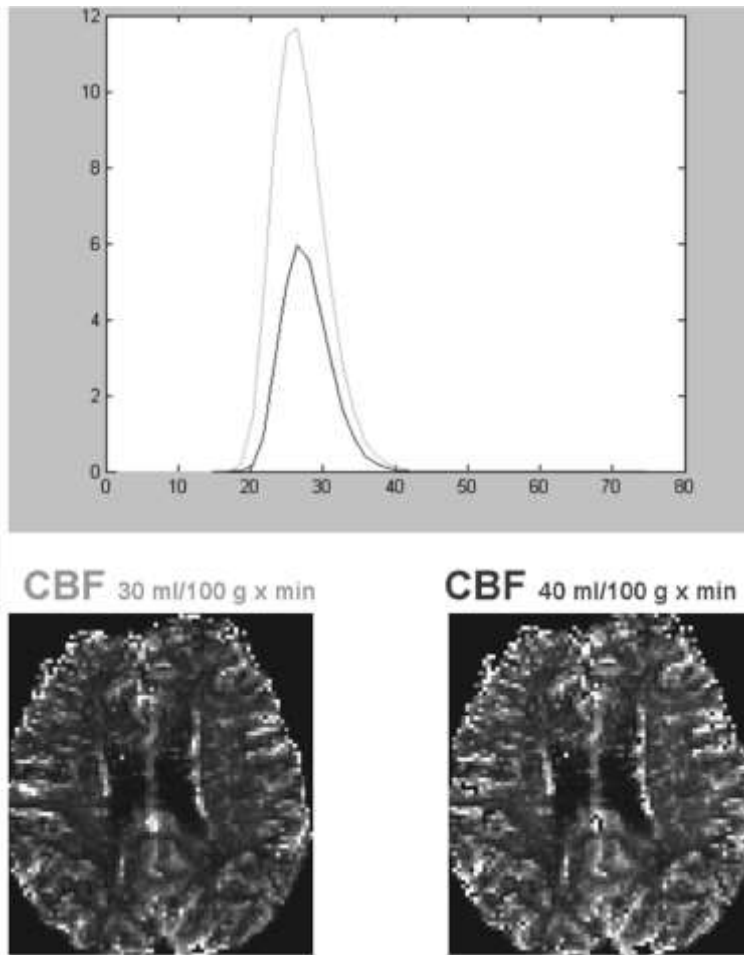


Figure 6. The graph shows two different shape of AIF obtained with a manual (light line) or automated (dark line) detection. The use of two different AIFs to process the same PWI row data with the same deconvolution method generates different maps of CBF.

ROLE OF ADVANCED IMAGING IN STROKE: WHAT WE LEARN FROM THE TRIALS IN 2015

Until 2015, intravenous tissue-type plasminogen activator (rtPA) has been the only proven and accepted reperfusion therapy in acute ischaemic stroke [65]. Recent clinical trials (MR CLEAN¹, ESCAPE², EXTEND-IA³, SWIFT PRIME⁴ and REVASCAT⁵) proved the

¹ Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischaemic Stroke in the Netherlands.

² Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion With Emphasis on Minimizing CT to Recanalization Times.

³ Extending the Time for Thrombolysis in Emergency Neurological Deficits_Intra-Arterial.

⁴ Solitaire With the Intention for Thrombectomy as Primary Endovascular Treatment.

⁵ Randomized Trial of Revascularization with Solitaire FR Device versus Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation Large Vessel Occlusion Presenting within Eight Hours of Symptom Onset.

efficacy of adding endovascular therapy to intravenous rtPA because of the large vessel occlusion in the anterior circulation. In particular, endovascular therapy with mechanical thrombectomy was associated with better functional outcomes than standard medical care with rtPA [66]. The overarching philosophy and new approach of these recent studies is to select patients with small ischaemic core at baseline and adequate collateral circles or salvageable brain [67].

Until now neuroimaging, especially advanced techniques, has been used to extend the therapeutic window for including a larger number of patients with stroke that could benefit from reperfusion. In these new trials, imaging was used to identify intracranial thrombi and measure the extent of salvageable brain in patients with a higher a priori probability of good outcome because of small ischaemic lesions and good collaterals. Unlike previous negative studies, probably both the technical improvement in materials adopted for endovascular thrombectomy and improved patient selection could have driven this change in outcome [68].

The demonstration of thrombectomy as an effective treatment implies that intracranial vessels should be evaluated in the acute phase together with NCCT. Although NCCT combined with a high score of National Institutes of Health Stroke Scale (>10) would be sufficient to select the right patients for thrombectomy, the majority of neuroradiologists prefers a patient selection based on NCCT and CTA.

In MR CLEAN [69] and in REVASCAT [70], the use of CT ASPECTS <7 or DWI ASPECTS <6 to exclude patients with large baseline infarcts demonstrated benefits of endovascular treatment (OR 1.66 and 1.57). Furthermore, a better outcome after thrombectomy was proved by the ESCAPE study (OR 2.53) [21] that added CTA for patient selection. Minimal or no pial collateral vessels in more than 50% of the ischaemic anterior circulation, corresponding to ASPECTS of 5 or less in patients with anterior circulation obstruction, was documented to be a predictor of poor outcome.

Another important observation coming from the most recent trials is the impact of penumbra imaging. The EXTEND IA study selected the eligible patients by using CTP. By using dedicated not commercial CTP software, they defined the infarct core as the tissue where the relative CBF was $<30\%$ of that in normal tissue, and the penumbra as the surrounding tissue with a time to maximum (Tmax) delay of more than 6s. When patients were selected using MR evaluation of the penumbra, the DWI-PWI mismatch was defined as a mismatch ratio >1.2 , with absolute mismatch volume $>10\text{ml}$ and ischaemic core lesion volume $<70\text{ml}$. The use of perfusion data to select patients for thrombectomy led to 71% of functional independence (mRS=0-2), a greater result than that achieved in other trials which used NCCT and CTA.

The role of advanced neuroimaging techniques in selecting patients for thrombectomy is currently a matter of discussion. There are two main approaches to the problem of patient selection based on MR measurement of the perfusion; they can be summarized in “physiology is brain” and “time is brain.”

The imaging of penumbra is supposed to monitor the progression of the pathophysiological cascade during stroke, and its utility in selecting patients for reperfusion can be exploited in different phases of the pathologic process. Up until now, the physiological imaging has been evaluated at a later time or beyond the therapeutic window, when the benefit margin is smaller [71]. On the contrary, the EXTENDED IA and SWIFT PRIME trials evaluated the role of physiological imaging for patient selection within 6 hours, when a beneficial treatment is more likely. This different approach could explain the better outcome

than that described by other trials without physiological imaging. A recent observational study [72] supports the hypothesis that the perfusion imaging-based selection can improve the clinical outcome; in fact, patients with target mismatch and small infarct core had a better outcome than patients without mismatch. Perfusion within the current standard time window for thrombolysis can identify patients that more likely benefit from intravenous rtPA and, among these, a subgroup with “target mismatch” that can not be identified using clinical/NCCT criteria alone.

The second approach to the imaging based patient selection is pragmatic. It is based on imaging methods to exclude contraindication to treatment; furthermore, the imaging has to be performed as fast as possible because benefits from thrombolysis decrease over time. This pragmatic approach expects that NCCT and CTA findings (small core, proximal vessel occlusion, and good collateral vessels) are likely to lead to the same clinical decision as a CT perfusion-based paradigm. For this reason, it is not ethical to perform adjunctive diagnostic tests when a beneficial treatment is already available. The combination of NCCT and CTA is the most pragmatic approach used in the recent endovascular trials according to the Bayesian view [73] of the decision-making: an adjunctive diagnostic test in patient selection is justified if it changes the therapeutic decision. NCCT and CTA allow the exclusion of haemorrhage and the depiction of large vessel intraluminal thrombus, thus changing the therapeutic decision to re-perfuse; additional imaging techniques, such as CTP or MR perfusion, do not. This point of view criticizes the improved outcome of trials adopting a perfusion-based patient selection by contending that perfusion led to exclusion of several patients (25% in EXTEND IA) who probably would have been enrolled for thrombectomy if selected by using NCCT and CTA alone. Moreover, a re-evaluation of patients of MR CLEAN trial demonstrated that perfusion is useful and revealed that patients with mismatch >1.2 or ischaemic core <70 ml have a better outcome than other patients. However, perfusion can not exclude patients from treatment because patients with no mismatch or large ischaemic core also benefit from thrombectomy [69].

After the publication of successful endovascular treatment, NCCT has to be used to exclude intracranial hemorrhage and to estimate the infarct core size by means of ASPECTS. The vascular imaging with CTA or multiphase CTA is fundamental to assess vascular occlusion and obtain information on collateral flow.

CTP may not be necessary in the first 6 hours from onset where the efficacy of endovascular treatment is clearly proven but it can increase confidence in the assessment of the infarct core [74]. If we use more precise (specific) imaging such as perfusion, will we lose sensitivity and exclude patients who might benefit from treatment? This is the radiological question and it is still open [75].

ROLE OF ADVANCED IMAGING IN STROKE: FUTURE PERSPECTIVE

Imaging in acute stroke for patient selection has to be readily available in a stroke centre on a 24/7 basis. The best imaging measures for planning intravenous rtPA or endovascular thrombectomy are NCCT and CTA.

Advanced techniques for penumbra assessment could be introduced in an experimental protocol if they do not unduly delay the treatment. However, the volumes of infarct core and

ischaemic penumbra as predictors of clinical outcome are under judgment because of conflicting results coming from observational studies. The diagnostic and clinical utility of perfusion for patient selection has not been proven in controlled studies of adequate power [26]. Randomized controlled studies are necessary to introduce MR or CT perfusion in the clinical practice in order to establish if perfusion-based selection allows a better outcome within or beyond 6 hours and the exclusion of patients that do not benefit from reperfusion.

Advanced neuroimaging methods for patient selection can be applied with two different strategies. On one hand, they can be applied within the therapeutic window in order to select the subgroup of patients with the best response to therapy; in this case, it must be acknowledged that, probably, not all patients who may benefit from therapy would be treated (selective strategy). On the other hand, they can be applied beyond the therapeutic window in order to select a larger number of patients (inclusive strategy); in this case, probably more people would benefit from treatment but there would also be a greater numbers of futile recanalization. The planning of future studies has to consider these two strategies: to select less people for a better outcome at a single-subject level or to select more people for a better outcome at the population level.

According to the current guidelines, the time window for thrombolytic therapy (<4.5 h) is still a limitation for an extensive treatment of patients with stroke. About 15-25% of strokes manifest at wake-up [76] and a large number of patients with acute stroke are admitted to hospital with unknown time of onset of stroke or beyond the therapeutic window. Assuming that patients with a relatively small infarct core surrounded by a large penumbra, currently excluded from treatment because of the therapeutic window, might benefit from therapies, further studies will be necessary to assess the role of advanced neuroimaging techniques as markers of penumbra and in the selection of patients to recanalise (imaging-based selection rather than time-based selection). Pursuant to this, an imaging-based selection to extend the therapeutic window or enlarge the sample of patients who might benefit from late reperfusion could be based on the mismatch between FLAIR and DWI [17].

CONCLUSION

- 1) The application of multimodal imaging is limited by the Bayesian view of “time is brain” in the first six hours from stroke onset.
- 2) Multimodal imaging could have a role in the first six hours to select patients for intravenous rtPA administration.
- 3) Multimodal imaging could have a role in extending the therapeutic windowing after six hours or in wake-up strokes.
- 4) The evaluation of patient selection based on quantitative imaging is recommended in a randomized controlled trial.

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Chapter 33

STROKE AND ATRIAL FIBRILLATION

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ABSTRACT

Atrial Fibrillation (AF) is the most common cardiac arrhythmia. Although clinical presentation may strongly differ and symptoms may be absolutely absent in several patients, the presence of AF carries on a significant impact on morbidity and mortality. Patients suffering AF have a five-fold higher risk of ischaemic stroke. The risk of clinical events has therefore to be scored in AF patients, in presence of high risk anticoagulation therapy is mandatory.

The best approach to this problem should be the elimination of AF; unfortunately this goal cannot be achieved in a significant percentage of patients. Antiarrhythmic drugs are often ineffective or induce side effects. Catheter ablation is effective in a high percentage of patients in presence of paroxysmal AF, but this procedure doesn't currently reach satisfactory results in persistent and long lasting forms. Nevertheless in patients with high risk of stroke anticoagulation should be delivered even after successful ablation.

Currently alternative therapies may improve the results of Vitamin K Antagonist (VKA) drugs. Novel Oral AntiCoagulants (NOACs) may provide a superior effectiveness and safety in comparison to VKAs. In addition, in patients with contraindications to anticoagulation or high risk of bleeding, the percutaneous occlusion of the Left Atrial Appendage may be an effective solution.

Keywords atrial fibrillation, stroke, anticoagulation, left atrial appendage closure, catheter ablation

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INTRODUCTION

Atrial Fibrillation (AF) is the most common cardiac arrhythmia [1]. In the western countries it is the most common cardiological cause of access to the Emergency Care and hospitalization [2].

Fibrillation means that the electrical activity of the atrial myocytes is completely uncoordinated, due to chaotic depolarizations where wavefronts move into the atria. Since the control and coordination of the sinus node is lacking, multiple impulses reach the atrio-ventricular node, thus inducing ventricular beats with irregular intervals and, usually, high rate.

The prevalence of AF in the overall population is not negligible. The incidence of AF episodes rises accordingly with the age and the arrhythmia is common in the elderly, particularly in presence of heart disease. Valvular diseases (mainly mitral valve pathology), heart failure and hypertensive cardiomyopathy significantly increase the risk of AF. Nevertheless AF may occur even in young people, in absence of any underlying heart disease. In the age decades up to 40 years AF is very uncommon; the incidence rises in the decades 40 to 60 years and in people aged over 70 years the prevalence of AF is estimated to be as high as 12% and rise over 20% in people aged more than 80 years. According with the improvement of life expectancy over time, the number of patients with AF in the overall population is getting greater and greater. In the USA the prevalence of AF in 2010 was estimated to range between 2.7 to 6.1 millions of people and is expected to rise up to 10 million in 2050 [3].

From a clinical point of view the impact of AF may range from absolutely asymptomatic to a very challenging condition. The most common symptom is palpitations due to the increase in heart rate and to the irregular heartbeats. Dyspnoea, dizziness and fatigue are common. The hemodynamic impact of AF is related to the underlying heart disease: usually well tolerated in normal hearts, it may precipitate heart failure and shock in patients with very depressed cardiac function. On the other hand many patients suffer of paroxysmal or persistent episodes of AF in absence of any symptom. It was observed that at the time of the diagnosis about 1 out of 3 patients affected by AF are absolutely asymptomatic and underwent medical examination for reasons not related to AF.

AF is classified as paroxysmal when any episode terminate spontaneously in less than 7 days, persistent when last longer or require a medical intervention to be terminated and permanent when it is impossible to restore the sinus rhythm or the attempt is judged to be useless due to the early recurrence of the arrhythmia.

A single AF episode or 2 may occur in healthy people during lifetime, usually in particular situations (overstress, alcohol or drug abuse, fever). The case of recurrent episodes implies that a mechanism inducing the arrhythmia is present and usually AF episodes are likely to increase over time. The time course of the disease usually shows the progressive transformation of paroxysmal AF forms into persistent and permanent ones. This mechanism is related to the possible progression of the underlying disease as well as the facilitating effect on AF due to the AF itself. Increasing burdens of AF were found to modify atrial refractoriness (facilitating the initiation and propagation of fibrillatory wavelets), to induce the prevalence of fibrous tissue into the atrial wall (increasing dyshomogeneity and decreasing mechanical compliance) and finally inducing a dilatation of the atrial size.

Experimental data clearly demonstrated that AF begets AF. Once the disease has induced such modifications in the atrial substrate, the clinical presentation usually progresses from paroxysmal to permanent.

CLINICAL IMPACT OF ATRIAL FIBRILLATION

Since the acute risk of AF is usually low and chronic arrhythmia may not significantly affect quality of life in most patients, in the past years AF was considered to be harmless. Most of the attention was addressed to the relief of symptoms, mainly by means of the control of cardiac rate and patients were usually reassured about the consequences of the arrhythmia.

Further data demonstrated that AF significantly impact on the outcome.

The presence of AF was estimated to three-fold increase the risk of symptomatic heart failure. Observational data report that in people with depressed cardiac function the presence of AF doubles the risk of major events and hospitalizations and significantly worsens the outcome.

In most of the longitudinal studies the presence of AF was found to double overall mortality. These data may reflect the higher prevalence of AF in patients affected by structural heart disease, nevertheless the presence of the arrhythmia was found to carry out significant morbidity and mortality.

Finally, AF was reported to increase the risk of clinical strokes as high as 5-fold, with a steep increase in percentage of strokes attributed to AF from 1.5% at ages 50 to 59 to 23.5% at ages 80 to 89 [4] Importantly, the clinical course of ischemic stroke associated with AF is often more severe than for strokes of other causes, further emphasizing the need for stroke prevention [5].

In addition, even in absence of clinically relevant episodes, a significant burden of AF episodes was demonstrated to induce asymptomatic strokes (as detected by TC or MRI scans) and may be responsible of dementia.

Many of the patients referred to the Stroke Units suffer of AF, often undertreated and in many cases underdiagnosed. It is estimated that at least 15% of the overall number of clinical strokes is related to AF. The neurological adverse events affect mortality and morbidity, and heavily impact on individual and social costs due to possible disabling outcome.

The relation between AF and ischaemic stroke was supposed to be related to arrhythmic episodes and to be time dependent. Since during AF the atrial wall is lacking of coordinated contraction, atrial systoles are missing. The velocity of blood flow into the atrial chambers is consequently reduced, peripheral portions as appendages or pouches into the walls may provide further reduction of flow thus leading to the formation of clots and thrombi that may in turn embolize, with significant clinical consequences when embolization occurs in the systemic circulation. The longer the duration of the arrhythmia the higher is the risk of embolization; conventionally, episodes lasting more than 48 hours are considered to be significantly risky.

The risk of stroke secondary to either paroxysmal or permanent AF is the same and, from the perspective of thromboprophylaxis, should be treated in the same manner [6]. This condition implies the need of anticoagulation therapy in order to reduce stroke events to the level of controls.

Table 1. CHA₂DS₂-VASc scoring system

	<i>Risk factor</i>	<i>Points</i>
C	Congestive heart failure	1
H	Hypertension	1
A2	Age $\geq$ 75 years	2
D	Diabetes mellitus	1
S2	History of stroke or transient ischemic attack or thromboembolism	2
V	History of vascular disease, either coronary or peripheral	1
A	Age 65-74 years	1
Sc	Sex category: Female	1

Many concomitant factors affect the risk of stroke in AF patients. The risk was proved to increase in the elderly, female gender is another risk factor. Congestive heart failure, hypertension and other systemic diseases such as diabetes and renal failure increase the risk of clinical strokes. All these factors have been included into score systems (CHADS₂, CHA₂DS₂-VASc) in order to carefully stratify the risk in individual patients and address the anticoagulation therapy [7]. Until recently, the CHADS₂ score [8] was the most commonly used, but it has been superseded by the CHA₂DS₂-VASc score [9]. The point system for this scoring system is shown in Table 1.

In contrast with CHADS₂, this updated system assigns 2 points for age over 75 years and accounts for stroke risk in the relatively younger group of patients (age 65–75) and in females, neither of whom were included in CHADS₂. The CHA₂DS₂-VASc score ranges between 0 and 9 with a respective estimated stroke risk of 0 to 15.2% per year. Note that for females who are younger than 65 years, no points are given for sex. The major advantage of the CHA₂DS₂-VASc score over the CHADS₂ score is that it is more accurate for lower-risk categories. It has been adopted in most of the recent guidelines that address stroke risk in AF.

Many concerns have been recently raised about most of these issues. First of all, in patients where AF is not permanent, embolization was supposed to be mainly related to sinus rhythm restoration. The recurrence of atrial contraction may mechanically displace thrombi from their sites into the appendages or adhesions to the endocardial walls. When embolization occurred later after an episode, the stunning of atrial contraction was identified as the cause of the late clinical event. Recent observations do not longer support this aetiology of clinical strokes. Data of cardiac monitoring provided by implanted Devices form rhythm management and implantable Loop Recorders demonstrated that a clear relation between AF and stroke episodes cannot be identified. Time relation between AF and stroke is absolutely variable and AF duration was not found to linearly correlate with the stroke risk. In addition in high risk patients (CHA₂DS₂-VASc score >4) stroke may occur even in absence of AF episodes.

According to these observations currently AF is no longer considered as a strict risk factor, but is going to be identified as a risk marker, to be combined with other concomitant factors that impact on the patient's risk profile.

The CHA₂DS₂-VASc score has been confirmed as a more effective method of stratifying stroke risk in patients with AF, compared to CHADS₂, and is able to identify truly low-risk patients who would not benefit from anticoagulation [10].

Current indications to anticoagulation therapy recommend to treat patients with high risk score even when AF is suppressed by specific therapy.

THERAPEUTICAL ISSUES

The overwhelming risk of stroke in AF patients, particularly when concomitant risk factors and morbidities are present, implies that any therapeutic option has to be used to reduce clinical events.

Mainstay of the therapy is stroke risk prevention and rhythm control.

The first step of this strategy is based on the diagnosis of AF.

As before mentioned many episodes of AF may be absolutely asymptomatic. Symptoms may be different in individual patients and may vary over time in each patient. Even in patients with typical symptoms during the arrhythmia, the incidence of asymptomatic may be as high as 50% of the true AF burden. Data from registries report that about in 30% of the patients AF is diagnosed in absence of symptoms; when episodes are frequent or persistent a significant percentage of the patients cannot adequately recognize the presence of AF, even if they were symptomatic in the past.

Actually, underdiagnosed AF, mainly in paroxysmal form, is very common.

In relation to the association with ischemic stroke, it is interesting to underline that AF may not be identified as the direct cause of the episode since a wide percentage of the patients have no longer AF during the hospitalization. History of palpitations may be not present or unreliable and even conventional monitoring may not identify the presence of paroxysmal episodes. Any effort in order to identify the presence of AF in this population should be recommended; currently the use of implantable Loop Recorder was found very effective and helpful.

ANTICOAGULATION THERAPY

The prevention of stroke is the mainstay of the treatment, either in case of acute episodes or in the follow-up of AF patients.

The use of anticoagulant therapy may prevent the increased stroke risk in AF patients. Guidelines clearly indicate the overwhelming importance of anticoagulant therapy in such patients. Nevertheless all the data from clinical practice report that usually about one half of the patients with indications to anticoagulation are not treated.

On the other hand the benefit of anticoagulation is actually faced by an increased the risk of bleeding induced by the therapy. A careful evaluation of the risk/benefit ratio is required in any individual patient.

In absence of chronic therapy, acute episodes are considered to raise no significant risk of embolism in the first 48 hours since the occurrence. It is then critical to reliably determine the time of onset of any episode. If it is impossible or when AF lasts longer than 48 hours, anticoagulation (mainly using heparin) is mandatory. In case of unstable clinical conditions, when urgent restoration of sinus rhythm is judged to be necessary, there is a general agreement about the use of Transoesophageal Echocardiography in order to assess the possible presence of thrombi into the Left Atrial Appendage (LAA).

In AF patients the risk is estimated according to the score. In male patients with a CHA₂DS₂VASc score of 0 and in most patients with a score of 1, the stroke risk is less than 1% per year, and it not significantly differs from controls. These patients are not likely to

benefit from anticoagulant therapy. They are usually approached on a case-by-case basis with careful assessment of bleeding risk and discussion of risks and benefits of anticoagulant strategies [11].

A 2% per year cut-off is usually used to identify high-risk patients in whom the risk of stroke significantly outweighs the risk of bleeding on anticoagulants. In general, patients with a CHA₂DS₂-VASc score equal to or greater than 2 have a greater than 2% stroke risk per year and are most likely to benefit from antithrombotic therapies [12].

The benefit of anticoagulant therapy is unfortunately balanced by the risk of bleeding induced by drugs. Even the risk of bleeding is assessed according to the clinical profile of the patients, taking in account comorbidities, which can increase the risk and lifestyle. A clinical score is currently used in order to stratify the risk profile in individual patients, the HAS-BLED score (Table 2). Interestingly, most of the parameters used in score systems evaluating the risk of stroke are included in the score system evaluating the bleeding risk; finally patients who are more likely to benefit by anticoagulation are more likely to have side effects during therapy.

Generally, a HAS-BLED score of 3 or greater indicates increased 1-year risk of intracranial bleed, bleeding requiring hospitalization, drop in hemoglobin of at least 2 g/dL, or need for transfusion.

One problem with the bleeding risk scores is that they were derived from studies that included bleeding events of different severity. Most bleeding events do not lead to death or severe disability with the exception of intracranial bleeding, which is, therefore, the primary concern when assessing bleeding risk.

The estimated bleeding risk with anticoagulant therapy ranges from 0.2% to 0.4% per year but could be much higher in patients with prior severe bleeding, intracranial hemorrhage, thrombocytopenia, coagulopathies, recent surgery, or ongoing bleeding, aortic dissection, malignant hypertension, and in those receiving a combination of anticoagulant and antiplatelet agents (i.e., patients affected by Ischemic Heart Disease, recipient of coronary stents).

Once the decision to initiate anticoagulation has been made, assessment of whether the patient is a suitable candidate for warfarin therapy is required.

Warfarin has been used for decades for stroke prevention. It remains the only acceptable anticoagulant in patients with valvular AF. Multiple randomized clinical trials have assessed the efficacy of warfarin for stroke prevention in patients with nonvalvular AF [13]. These trials demonstrated that warfarin significantly reduces stroke risk, stroke severity, and 30-day mortality compared with no anticoagulant therapy [14]. The net clinical benefit of warfarin increases the longer the patient remains within the therapeutic range achieving net benefit over antiplatelet therapy when the TTR is above 65–70%, with an optimal TTR defined as >70% in a recent European consensus document [15, 16].

Difficulty in achieving adequate control of anticoagulation with warfarin has been cited as a common barrier to its use [18]. A poor control of anticoagulation is correlated with increased bleeding and thromboembolic risk [19]. Indeed, it has been shown that when TTR falls below 50%, stroke outcomes are worse than if the patient remained untreated [20], and bleeding risk is higher [21].

The importance of achieving satisfactory control of anticoagulation rapidly after starting warfarin has been illustrated by studies examining the incidence of stroke in the first month

after warfarin initiation; this period was associated with a 71% increased relative risk of stroke, presumed to be due to the hypercoagulable state.

Another limitation with the medication is the dietary restriction on intake of vitamin K-rich green vegetables, which are emphasized as healthy food choices especially in patients with heart disease. Higher warfarin doses are required in patients who consume greens and salads. It is important that patients be consistent in their intake of vitamin K-rich foods to avoid labile INRs, a difficult task for most patients.

Finally, there are several drugs that might interact with warfarin and potentially interfere with its safety or efficacy. These drugs include amiodarone, statins including simvastatin and rosuvastatin (not atorvastatin or pravastatin), fibrates (fenofibrate, gemfibrozil), antibiotics (sulfamethoxazole/ trimethoprim, metronidazole), and azole antifungals (fluconazole, miconazole, voriconazole). The use of drugs that induce the cytochrome P450 enzyme CYP2C9, such as rifampin, decrease warfarin effectiveness by reducing INR values. Other non-CYP2C9-dependent drug interactions exist as well.

ASPIRIN MONOTHERAPY OR IN COMBINATION WITH OTHER AGENTS

Aspirin monotherapy or aspirin plus clopidogrel both increase the risk of bleeding without appreciable benefit, and, as such, their use for stroke prevention in patients with AF is not well supported. The combination of aspirin plus low-dose warfarin was assessed in the SPAF-III trial [22] that randomized AF patients at stroke risk to either aspirin plus low-dose warfarin or to dose-adjusted warfarin to target a therapeutic INR. In this trial, patients on aspirin plus low-dose warfarin had significantly higher morbidity and mortality than patients who took adjusted-dose warfarin alone. Thus, the combination of low-dose warfarin plus aspirin should not be used for stroke prevention in AF.

In contrast, the combination of aspirin plus full anticoagulation with warfarin has not been well studied. Limited post-hoc data from the SPORTIF trials suggest, however, that this combination does not reduce the risk of stroke or thromboembolism more than warfarin alone [23].

Table 2. The HASBLED score

	<i>Risk factor</i>	<i>Points</i>
H	Hypertension	1
A	Abnormal renal and liver function	1 or 2
S	Stroke	1
B	Bleeding	1
L	Labile INRs	1
E	Elderly (e.g., age >65 years)	1
D	Drugs or alcohol (1 point each)	1 or 2

NEW ORAL ANTICOAGULANT DRUGS

Anticoagulant options have increased substantially in the past few years with the introduction of Novel Oral AntiCoagulants (NOACs), including the direct thrombin-inhibitor dabigatran and the factor Xa inhibitors rivaroxaban, apixaban, and edoxaban (Figure 1).

A limitation with these agents (versus warfarin) is that patients are not therapeutically anticoagulated when they miss a dose, whereas missing a single dose of warfarin may not have the same effect.

Dabigatran. The value of dabigatran for prevention of stroke or thromboembolism in AF was tested in the RE-LY trial (Randomized Evaluation of Long-Term Anticoagulation Therapy) [24]. In this trial, 113 patients with AF at high risk for stroke were randomized to dabigatran (110 mg or 150 mg twice daily) or adjusted-dose warfarin (INR target 2.0–3.0). By intention-to-treat analysis, dabigatran 150 mg was superior to warfarin for stroke prevention. Importantly, the risk of intracranial or life-threatening bleeding was significantly lower for both dabigatran doses compared with warfarin. Of note, gastrointestinal bleeding was more common with dabigatran 150 mg than warfarin; rates were similar for dabigatran 110 mg versus warfarin.

Rivaroxaban. This factor-Xa inhibitor was assessed in ROCKET-AF (Rivaroxaban Once-daily oral direct factor Xa inhibition Compared with vitamin K antagonism for prevention of stroke and Embolism Trial in Atrial Fibrillation) [25]. In this trial, 14,264 patients with AF and at risk for stroke were randomized to rivaroxaban (20 mg once daily) or warfarin (INR target 2.5). In the warfarin arm, INR was in the therapeutic range only 55% of the time. Results showed rivaroxaban was not inferior, but not superior, to warfarin for the prevention of stroke or systemic thromboembolism, the primary end points. From a safety standpoint, the overall bleeding rates were similar in the treatment arms with less life-threatening (fatal or intracranial) hemorrhage events with rivaroxaban.

Apixaban. This factor-Xa inhibitor was tested for stroke prevention in AF in two separate clinical trials [26, 27]. In the AVERROES trial (Apixaban Versus Acetylsalicylic Acid to Prevent Strokes) [26], 599 patients with AF deemed “unsuitable” for warfarin were randomized to apixaban (5 mg twice daily) or aspirin (81–324 mg daily). The trial was terminated early due to superiority of apixaban in achieving the primary end point: occurrence of stroke or systemic embolism. Importantly, the risk of major bleeding appeared to be similar with apixaban versus aspirin.

The ARISTOTLE (Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation) trial [27] randomized 18,201 patients with AF and at least one additional risk factor for stroke to either apixaban 5 mg or warfarin (target INR 2.0–3.0). In this trial, apixaban was superior to warfarin for the prevention of stroke or systemic embolism, the primary efficacy end point. There also appeared to be a mortality benefit with apixaban versus warfarin. Importantly, the risk of major and intracranial bleeding, the primary safety outcomes, occurred at lower rates in the apixaban group.

All three of these oral anticoagulants require dose adjustment in patients with renal insufficiency and are contraindicated in patients with end-stage renal failure. They are not indicated in patients with valvular heart disease or a mechanical heart valves.

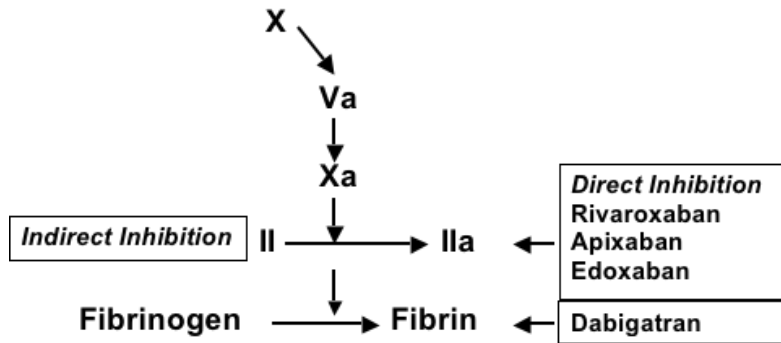


Figure 1. Mechanism and site of action of NOACs.

NON PHARMACOLOGICAL INTERVENTIONS

It is estimated that more than 90% of the thrombi rising into the left atrium form into the appendage. Non pharmacologic interventions have been developed to block the LAA to reduce the risk of stroke. These are especially valuable options for patients who are not candidates for chronic anticoagulation. In patients undergoing cardiac surgery [28], ligation to close the LAA has become a standard practice. Exclusion of the LAA cavity from the atrium can be achieved with devices that are positioned through an epicardial approach; however a thoracotomy approach is required. The introduction of less invasive catheter-based interventions to occlude the LAA has provided additional options.

Since the appendage is connected to the atrium by an orifice and (usually) a neck, the occlusion of the connection may result in total exclusion of blood flow from the appendage. Percutaneously positioning an occluding device in the appendage may obviate the need for sternotomy and cardiopulmonary bypass and is an attractive alternative to chronic anticoagulation with VKAs or NOACs in reducing stroke risk in non valvular AF.

LAA closure is an intervention of increasing interest, in particular for patients with either contraindications to anticoagulation therapy, embolic events while receiving anticoagulation therapy, or intolerance to anticoagulants.

Published trials demonstrate high overall success rates with favorable safety profiles compared with VKAs. Several device-related aspects still limit this approach, such as the high rate of incomplete LAA closure reported at long-term follow-up with transesophageal echocardiography and the occurrence of device embolization and migration.

Watchman device. The Watchman implant, a closure device that blocks the LAA, was recently approved by the US Food and Drug Administration (FDA) for stroke prevention in patients with non valvular AF who are at increased risk for stroke based on a CHA₂DS₂-VASc score of 2 or greater; candidates also must have an appropriate rationale for non pharmacologic therapy. The expandable-cage device is surgically delivered into the LAA, which subsequently endothelializes and isolates the LAA. Therapeutic warfarin is required for a minimum of 45 days after implant followed by aspirin and clopidogrel for 6 months and then aspirin alone.

This device was initially tested in the PROTECT AF (Watchman Left Atrial Appendage System for Embolic PROTECTION in Patients with Atrial Fibrillation) trial, a non inferiority

trial that randomized patients to either device implant or warfarin [29]. Device implant was successful in 91% of patients in whom it was attempted. Overall, the study showed non inferiority of the device to warfarin in terms of the primary efficacy standpoint, which included stroke, systemic embolism, and cardiovascular death; however, this came at the expense of higher incidence of procedure-related complications, which seemed to be dependent on a learning curve with the device.

In the PREVAIL (Evaluation of the Watchman LAA Closure Device in Patients with Atrial Fibrillation Versus Long-Term Warfarin Therapy) trial [30], although non inferiority was not achieved, the event rates were low and the safety of the procedure was much improved. This was also demonstrated in registry data, which showed improved safety with device implantation with increased operator experience.

Of note, both of these trials included only patients who were eligible for warfarin. Another nonrandomized study assessed the use of this device in patients with a contraindication to long-term anticoagulation. Results showed a very low incidence of stroke, which was lower than CHADS₂-matched controls taking either aspirin or clopidogrel [31].

Amplatzer Occluder. This device is similar to the system used to percutaneously occlude the Patent Foramen Ovale. It consists of two leaflets, connected by a shaft. When the device is deployed any leaflet approach to the edge of the ostium of the appendage, thus occluding the cavity itself. The design of this occluder may be helpful in presence of LAAs with limited longitudinal length, preventing the use of longer devices such as Watchman.

Data about the clinical use of the device are limited and most of them are provided by registries, thus clinical evidence of the results in a long term follow up is currently poor.

Lariat system. The Lariat system is another percutaneous system for occlusion of the LAA. This device, which requires both atrial transseptal and epicardial access, ligates the appendage from the pericardial space. While some small studies have shown it safe and efficacious in patients with AF who cannot take anticoagulation [32], other reports have not been as encouraging [33]. The device has been approved by the FDA for “soft tissue approximation”; it is not approved for stroke prevention.

Future refinements of LAA occlusion technology are expected to improve the feasibility of the procedure. The device design and coating should aim to overcome the limitations of incomplete LAA orifice sealing and device dislodgement. In contrast, acute procedural complications have been shown to be influenced by a learning curve, and operator experience deeply affects outcomes of the procedure [34]. Due to the skills and experience in transeptal puncture and navigation into the left atrium, Interventional Arrhythmologist are considered the recommended operators for this procedure.

One caveat is that there is a residual risk of stroke after undergoing LAA occlusion because the same risk factors for stroke in AF contribute to stroke from atherothrombosis and atheroembolism; also, thrombi can form in the body of the left atrium.

RHYTHM CONTROL: DRUGS AND RADIOFREQUENCY CATHETER ABLATION

In order to solve this clinical problem the most direct and effective approach could be to stop and (more) to prevent AF.

In the past the only possible approach was the use of AntiArrhythmic (AA) drugs. It is our practice to use propafenone, flecainide, sotalol, and dronedarone as first-line therapies in patients without structural heart disease [35]. Amiodarone is the second choice because of the risk of non cardiovascular toxicities with the latter. We occasionally choose amiodarone as a first-line therapy in elderly patients without structural heart disease for whom long-duration therapy and therefore side effects are unlikely. Patients with AF and congestive heart failure are treated with amiodarone.

Unfortunately the use of AA is often unsuccessful. Most of the data in literature report an unsatisfactory success rate of the AA drug therapy, either in interruption of acute AF episodes or in preventing recurrences. In addition secondary effects of many AA drugs advise against their use in patients with structural heart disease, thus limiting the possible choices. The most effective AA drug is Amiodarone. This drug can be used in patients with significant heart disease, mainly Coronary Artery disease and Heart Failure. This advantages are widely counterbalanced by the high incidence of significant side effects, limiting the clinical use. It was estimated that about one half of the patients receiving Amiodarone have therapy suspended in a 5 years period due to serious side effects. The disappointing long term results of drug therapy raised the interest about interventional therapies for AF.

Catheter Ablation is a percutaneous intervention aiming to modify the substrate of arrhythmias by performing thermic lesion in specific areas of the cardiac walls critically involved and responsible of the mechanism of the arrhythmia.

In the case of AF the arrhythmia is started by atrial ectopic beats (trigger) inducing desynchronization of the electrical activity of the atrium. The depolarization turns on a series of wavelets, moving into the atrium according to the possibility to find tissue to depolarize. Atrial size, dispersion of refractoriness, areas of scar tissue and orifices of vessels are all factors impacting on the maintenance of AF (substrate).

EP mapping of the atria demonstrated that most of the triggers are located into the Pulmonary Veins (PVs), were frequently repetitive ectopies (firing) were found to start AF episodes in paroxysmal forms.

Ablation into the antrum of PVs ostium may isolate the inner part of the vein, thus eliminating the trigger of AF episodes. The initial results of catheter ablation for AF were encouraging, thus spreading the indication to the procedure. Currently many and many patients affected by paroxysmal AF undergo ablation procedures.

Much more complex and honestly unreliable is the modification of the substrate. Since too many factors impact on substrate and no standardized workflow for substrate modification was clearly identified till to date, the ablation approach to this targets results both more complex and less effective in the long term. Ablation strategies adding further set of lesions to PVs isolation have been used. Linear lesions connecting PV isolation each other and to the mitral annulus or ablation of areas of abnormal electrical activity have been extensively tested in patients with persistent AF. Unfortunately long term results are still disappointing; the only multicentre randomized clinical trial performed to evaluate the best ablation strategy in such settings (STAR-AF2) was unable to demonstrate the superiority of extensive lesions in comparison to PVs isolation. Further investigations are required about this issue.

According to these observations Ablation techniques are effective in a significant percentage of patients affected by paroxysmal AF (where the trigger plays a key role) where success rate in the mid term follow-up is estimated to be as high as 75% off of antiarrhythmic

drugs. These results lead to consider ablation as a first line therapy in patients with paroxysmal clinical form and absence of significant structural heart disease (Figure 2).

The randomized MANTRA-PAF (Medical ANtiarrhythmic Treatment or Radiofrequency Ablation in Paroxysmal Atrial Fibrillation) [37] trial compared catheter ablation of AF to antiarrhythmic drug therapy as a first-line rhythm control intervention in 294 patients. At 24-month follow-up, significantly more patients in the ablation group were free from any AF and symptomatic AF. Quality of life was significantly better in the ablation group at 12 and 24 months. However, total AF burden was not significantly different between both groups. Similar information has emerged from the results of the RAAFT II (Radiofrequency Ablation for Atrial Fibrillation Trial) [38].

It is reasonable to recommend catheter ablation as first-line therapy for AF rhythm control in selected patients, i.e., those with paroxysmal AF preferring interventional treatment with a low risk profile for procedure-associated complications.

In presence of persistent or long lasting persistent AF (where the substrate is critically important and much more represented) the results of ablation in the real word is reported to be significantly less (about 50%, multiple procedures required).

In AF patients with CHA₂DS₂-VASc score ≥ 1 , catheter ablation of AF reduced the risk of the total/cardiovascular mortality and total vascular events. Atrial fibrillation recurrence predicts long-term cardiovascular outcomes, as well as the CHA₂DS₂-VASc score [39].

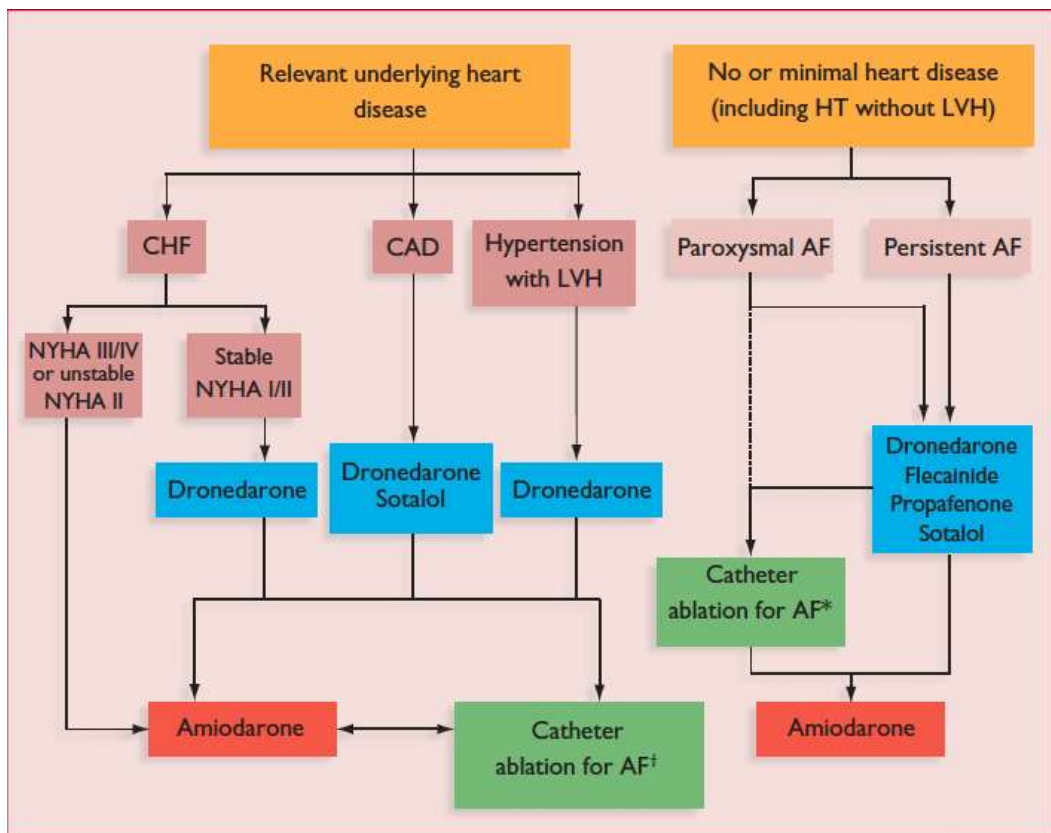


Figure 2. Flow chart for the therapy for suppression of AF, ESC Guidelines, 2012 [36].

Cerebrovascular complications related to catheter ablation of AF are relatively infrequent and typically occur early either during the procedure or within the first 24 h after AF ablation. Previous ischaemic stroke, peripheral vascular disease, mechanical valve replacement, and CHA₂DS₂-VASc score ≥ 3 are independent predictors of such complications. The majority of these events are ischaemic stroke with a benign clinical outcome, while intracranial hemorrhage may correlate with poor prognosis [40].

CONCLUSION

The most common serious complications of AF are stroke and thromboembolism. Medical and interventional therapies have been developed to prevent these complications. In patients with an estimated thromboembolic risk of greater than 1% to 2% per year, anticoagulation is warranted to reduce that risk and therapy is therefore strongly indicated. The recently introduced novel oral anticoagulants have been found to be at least non inferior to warfarin and, for some agents, to be superior to warfarin for the prevention of stroke and thromboembolism. In patients affected by AF with contraindications to anticoagulant therapy (mainly due to an increased risk of hemorrhage) percutaneous occlusion of the LAA is offered.

Rhythm control may significantly reduce the AF burden and subsequently the need of stroke prevention, at least in patients with no or minimal stroke risk profile. Catheter ablation is currently the most attractive option in younger patients and paroxysmal AF forms.

Clinical decision-making for stroke prevention in AF remains challenging in some patients, so a multidisciplinary approach for stroke prevention in AF is necessary. This model allows a complete assessment of patients' individual risks and ultimately facilitates clinical decision-making.

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Chapter 34

SUBCLINICAL ATRIAL FIBRILLATION (SCAF) AND EMBOLIC STROKE OF UNDETERMINED SOURCE (ESUS)

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ABSTRACT

Assessment of etiopathogenesis of ischemic stroke is essential to guide proper secondary prevention and reduce the risk of early recurrence. In spite of extensive diagnostic work-up, in about one-third of cases evaluated in routine clinical practice etiopathogenesis remains elusive (cryptogenic ischemic stroke, CIS). Thus CIS represents a very interesting arena for researchers, offering challenges and opportunities. Among the several potential causes underlying CIS, subclinical atrial fibrillation (SCAF) is one the most frequent and debated. On the other hand, the new clinical construct of embolic stroke of undetermined source (ESUS) has been recently proposed as an attempt to pragmatically overcome CIS conundrum. In both issues (SCAF and ESUS), diagnostic efforts are warranted, as they may provide the basis for trials evaluating potential benefit of anticoagulant therapy in such patients.

Keywords: subclinical atrial fibrillation, embolic stroke of undetermined source, stroke etiopathogenesis, anticoagulant therapy

INTRODUCTION

In the acute phase of ischemic stroke, assessment of etiopathogenesis is essential to implement early and proper secondary prevention therapy. To this purpose, several classification systems have been developed during time, such as Trial of Org 10172 in acute stroke treatment (TOAST) criteria [1], Causative Classification System (CCS) [2], Chinese Ischemic Stroke Subclassification (CISS) [3], Atherosclerosis-Small vessel disease-Cardiac

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source-Other cause (A-S-C-O) system [4] and Atherosclerosis-Small vessel disease-Cardiac source-Other cause-Dissection (A-S-C-O-D) system [5]. In synthesis, while about two thirds of cases of ischemic stroke are due to a well-defined cause (large vessel atherosclerosis; cardioembolism; small vessel disease; rare causes, e.g., dissection, vasculitis or hematologic disease), in the remaining third, called “cryptogenic ischemic stroke” (CIS), diagnosis remains elusive [6]. Apart from patients with an incomplete diagnostic evaluation and more than one possible etiology (e.g., atrial fibrillation and concomitant large vessel atherosclerosis), most of CIS lack of a determined cause despite extensive evaluation.

As for antithrombotic treatment, Warfarin–Aspirin Recurrent Stroke Study - WARSS [7] randomized patients with non-cardioembolic stroke (including CIS) to warfarin (at a dose adjusted to produce an international normalized ratio of 1.4 to 2.8) versus aspirin (325 mg per day). At two years follow-up, Authors found no significant differences between aspirin and warfarin in the prevention of recurrent ischemic stroke, mortality or in the rate of major hemorrhage. Rate of minor hemorrhage favoured antiplatelet therapy, instead. However, in post-hoc analyses of the cryptogenic subgroup, warfarin was associated with worse outcomes among patients with moderate stroke severity and better outcomes among those without baseline hypertension or with posterior circulation infarcts sparing the brainstem [8]. Moreover, in a cohort of patients with non cardioembolic stroke enrolled in such study, elevated pro-B-type natriuretic peptide concentration (which is known to be associated with atrial fibrillation and heart failure) identified a subgroup of cases (about 5% of the cohort) which benefitted more from anticoagulants than antiplatelet agents [9].

As recurrence rates after CIS may be even higher than those of large artery and cardioembolic causes [10], update and refinement in both diagnosis and treatment are warranted. To afford this relevant issue, two strategies have been recently proposed. On one hand, use of advanced diagnostic techniques (e.g., cardiac telemonitoring, D-dimer/cancer screening, transesophageal echocardiography, coronary computed tomographic angiography, advanced brain and vascular imaging, neurosonological techniques) may reduce the proportion of CIS and lead to a “tailored” therapy in specific subgroups of patients. Commonly underdetected conditions include subclinical atrial fibrillation (SCAF), cancer-related coagulopathy, paradoxical/aortogenic embolism, arterogenic embolism and branch occlusive disease [11]. On the other hand, basing on the assumption that most of CIS are embolic, the pragmatic clinical construct of embolic stroke of undetermined source (ESUS) has been created as the basis for future randomized trials for secondary prevention [12]. A detailed discussion of all the potential causes of CIS is beyond the aim of this chapter, which focuses on SCAF and ESUS.

SUBCLINICAL ATRIAL FIBRILLATION (SCAF) AND CRYPTOGENIC ISCHEMIC STROKE (CIS): DATA AND CHALLENGES

Detection of SCAF: Try and Catch It!

SCAF may be the cause of stroke in almost a part of patients with CIS [13]. Underdetection of SCAF remains problematic given its unreliable symptom profile, coupled

with its intermittent nature. Prolonged electrocardiographic monitoring may identify SCAF in a subset of strokes initially categorized as cryptogenic. As oral anticoagulant therapy (OAT) is indicated for secondary prevention in cases with ischemic stroke and documented atrial fibrillation (AF, regardless of whether it is paroxysmal, persistent or permanent) [14], detection of SCAF in patients with CIS may identify further candidates to OAT (see also following sections). On these premises, diagnostic strategies to improve detection of SCAF appear justified.

Current American guidelines about early management of patients with acute ischemic stroke recommend that cardiac monitoring should be performed for at least the first 24 hours [15]. However, AF-detection rate of 24-hour Holter monitoring seems not to be sufficient, being inferior even to serial ECG for 3 days [16]. Recent technological advances have allowed to perform long-term cardiac rhythm monitoring up to months or even years after a stroke [17]. When compared with 24-hour Holter monitoring, higher rates of AF detection with prolonged in-hospital [18-22] and outpatient monitoring (even with insertable loop recorders) [13, 23-30] have been reported.

Recently, two prospective, multi-center trials have shed further light on such issue: 30-Day Cardiac Event Monitor Belt for Recording Atrial Fibrillation after a Cerebral Ischemic Event (EMBRACE) [31] and Cryptogenic Stroke and Underlying Atrial Fibrillation (CRYSTAL-AF) [32]. In the EMBRACE trial, 572 patients were randomized to either a 30-day external event-triggered loop recorder or conventional evaluation via 24-hour Holter monitor. The primary outcome was detection of AF lasting 30 seconds or longer within 90 days. The detection of AF was significantly higher in the 30-day monitoring group than in the conventional Holter monitoring group (16.1% vs 3.2%, $p < 0.001$, respectively); detection of AF lasting longer than 2.5 minutes was also greater (9.9% vs 2.5%, $p < 0.001$, respectively). In addition, in the intervention group detection rate was higher within the first 3 months after index event than afterwards (18.5% vs 9%, respectively). The CRYSTAL-AF trial introduced an internal monitoring device; 441 patients were randomized to either a insertable cardiac monitor (ICM) or standard of care with intermittent outpatient electrocardiogram monitoring. At 6, 12, and 36 months, detection rates were significantly higher in the intervention group compared to standard monitoring (8.9% vs 1.4%, $p < 0.001$; 12.4% vs 2%, $p < 0.001$; 30% vs 3%, $p < 0.001$; respectively). At 12 months, among the patients in the intervention group in whom AF had been detected, maximum AF burden resulted >6 minutes in 1 day in 92% of cases and >12 hours in 1 day in 46% of cases. Of note, EMBRACE and CRYSTAL-AF trial presented several relevant differences. First of all, type of device: noninvasive external loop recorder in EMBRACE is subject to participant compliance and may underestimate actual AF detection rates. Moreover, mean time from event to randomization: 75 vs 38 days after the stroke or TIA, respectively; of course, AF occurring prior to randomization was missed. Finally, time of monitoring: 3 vs 12 months, respectively. Despite these differences, the detection rate in the EMBRACE trial was higher, maybe due to mean age (72 vs 61 years, respectively), selection criteria (stroke evaluation was less rigorous in EMBRACE trial) and devices' detection thresholds (>30 seconds vs 2 minutes, respectively) [33].

Taken together, the above mentioned data suggest that detection rate of SCAF through long-term heart rhythm monitoring systems, which are relatively invasive and expensive, is relatively low, raising issues of cost-effectiveness [34]. Thus, a simple and cheap selection tool able to identify patients with CIS at high risk of SCAF, who would represent ideal candidates for monitoring, seems needed. To date, various predictive scores of SCAF based

on several (and sometimes complex) clinical, instrumental, laboratory and genetic factors, have been developed [9, 23, 35-43]. A detailed evaluation of routine neuroradiologic investigations, which may give relevant hints of cardioembolism (such as early hemorrhagic transformation of brain infarction [44]), has been recently included in the “cardioembolic profile” elaborated by our group [45]. In particular, considering commonly available data, we identified age older than 75 years, female sex, left atrial dilation, cortical–subcortical cerebral index infarct, ischemic lesions in multiple vascular grounds, and spontaneous hemorrhagic transformation of brain infarction as significant predictors of cardioembolic stroke due to atrial fibrillation. All of the above mentioned scores present relevant strengths and limitations and need further validation in large population before wide implementation.

SCAF and Stroke Pathogenesis: Cause or Marker?

One of the most relevant studies showing the association between SCAF and ischemic stroke was the Asymptomatic Atrial Fibrillation and Stroke Evaluation in Pacemaker Patients and the Atrial Fibrillation Reduction Atrial Pacing Trial (ASSERT) [46]. 2580 patients, aged >65 years, with hypertension and no history of atrial fibrillation, in whom a pacemaker or defibrillator had recently been implanted, were monitored for 3 months. Monitoring aimed to detect subclinical atrial tachyarrhythmias, defined as episodes of atrial rate >190 beats per minute for more than 6 minutes. Previous studies had indicated that, depending on the programming of the pacemaker, the detection of such atrial high rate episodes (AHRE) correlated well with electrocardiographic documentation of atrial fibrillation. [47]. Patients were followed for a mean of 2.5 years for the primary outcome of ischemic stroke or systemic embolism. By 3 months, subclinical atrial tachyarrhythmias detected by implanted devices had occurred in 261 patients (10.1%). Such arrhythmias resulted associated with an increased risk of clinical atrial fibrillation (hazard ratio, 5.56; 95% confidence interval [CI], 3.78 to 8.17; $P < 0.001$) and of ischemic stroke or systemic embolism (hazard ratio, 2.49; 95% CI, 1.28 to 4.85; $P = 0.007$), even after adjustment for predictors of stroke (hazard ratio, 2.50; 95% CI, 1.28 to 4.89; $P = 0.008$). Of 51 patients who had a primary outcome event, 11 had had subclinical atrial tachyarrhythmias detected by 3 months, and none had had clinical atrial fibrillation by 3 months.

ASSERT greatly contributed to renew interest in the arena of SCAF and stroke, which remains characterized by intriguing critical issues. First of all, cautious interpretations of device-detected arrhythmias are needed. Although AHREs have been used as a surrogate for AF, ASSERT investigators evaluated the positive predictive value of AHREs for intracardiac electrograms-confirmed AF and found that in 17.3% of cases AHREs were falsely positive [48]. Such results were predominantly due to repetitive non-re-entrant ventriculoatrial synchrony, also known as atrioventricular desynchronization arrhythmia.

Moreover, reported thresholds of episode duration (and their clinical relevance) are highly variable and debated [49-50]. SCAF duration ranges from 30 seconds or less (see previous sections; [51]) to 5 minutes (with 2.8 greater risk for stroke or death) [52, 53] and 24 hours (with 3.1 greater risk for thromboembolism) [54]. Likewise, a daily burden of 3.8 hours [55] and 5.5 hours [53] has also been associated with significant increases in the risk of stroke (9 and 2-fold, respectively). Several authors have attempted to integrate commonly used clinical scores (CHADS2 and CHA2DS2VASC [56, 57]) with AF episode duration/burden in

order to refine stroke risk stratification. In particular, Botto et al. [58] found a low risk of stroke (0.8%) in the following subgroups: patients with <5 minutes of AF and a CHADS2 score <2; AF lasting 5 minutes to 24 hours with a CHADS2 score <1; AF lasting >24 hours with a CHADS2 score of 0. In another paper [59], the integration of AF presence/duration/burden improved the c-statistic from 0.653 to 0.713 for CHADS2 and 0.898 to 0.910 for CHA2DS2VASc.

On top of that, even if SCAF resulted associated with an increased risk of stroke and embolism in ASSERT, a recent analysis of the study showed that very few patients had SCAF in the month before their event [60]. In particular, SCAF was detected in 26/51 patients who experienced stroke or systemic embolism during follow-up; among them, 18/26 had SCAF before stroke or systemic embolism, but only 4/18 within 30 days. The remaining 14/18 had SCAF >30 days before the event, with the most recent episode occurring at a median interval of 339 days earlier. In 8/26 cases SCAF was detected only after their stroke despite continuous monitoring (for a median duration of 228 days) before their event. Furthermore, most SCAF occurring before embolic events was far shorter than 48 hours in duration, which is commonly believed to be the minimum duration required for thrombus to form in the left atrial appendage before cardioversion [61]. Basing on both duration of episodes of SCAF and temporal relationship between SCAF and stroke, the role of SCAF in stroke pathogenesis has been called into question. Of note, among Virchow's triad (stasis, hypercoagulability, vessel wall abnormalities), prolonged (>48 hours) atrial stasis due to concomitant AF shouldn't be "conventionally" considered determinant for thromboembolism [62]. As for ASSERT patients, apart from potential non-AF related causes of stroke (such as lacunar stroke due to small vessel disease), some "non-conventional" explanations of AF-related stroke have been postulated [60]. First of all, mechanical atrial stunning after cardioversion (lasting for hours to days after electric cardioversion, as showed in echocardiographic studies [63]) may explain a delay between AF and a presumed cardioembolism. Even more interestingly, SCAF itself may not represent a cause embolism but rather a marker of "atrial cardiomyopathy" leading to embolism. AF may cause changes in atrial structure and endothelial function [64]; furthermore, it is associated with biochemical evidence of inflammation [65], markers of hypercoagulability [66] and left atrial spontaneous echo contrast [67]. In other terms, even short episodes of SCAF might trigger chronic modifications in the atria that lead to thrombus formation sometime after the actual occurrence of SCAF. On the other hand, "atrial cardiomyopathy" as a cardiac source of emboli has been reported even independently from AF. In particular, Kamel et al. found an association between baseline P-wave morphology (as expression of atrial abnormalities), incident stroke [68] and MRI-detected vascular brain injury [69], even after accounting for AF. Moreover, De Bruijn et al. [70] detected left atrial appendage (LAA) thrombus in 16% of patients with transient ischemic attack or ischemic stroke without definite cause undergoing transesophageal echocardiography. Finally, LAA volume and morphology may contribute to thrombogenesis, too. Taina et al. [71] found higher LAA volumes in patients with cryptogenic stroke patients (vs control subjects). As for LAA morphology (even if considered in patients with atrial fibrillation), "cactus," "windsock" and "cauliflower" were reported to correlate with the risk of stroke (4.08, 4.50 and 8.0 folds higher, respectively, in comparison to "chicken wing") [72]. Of note, in cases of "atrial cardiomyopathy without AF," episodes of SCAF may have been missed (depending on method of detection) and/or SCAF/AF may appear only in the late stages of atrial disease [73].

Device-Detected SCAF and Therapy: To Anticoagulate or Not to Anticoagulate?

Oral anticoagulant therapy (OAT) in patients with “conventional” AF not only reduces stroke risk [14], but also stroke severity or death [74-76], so that it is indicated (according to clinical status, evaluated by CHADS2 and CHADSVASC2 score) by international guidelines [56, 57, 77, 78]. In comparison to “conventional” AF, strong evidence about the benefit of anticoagulation in patients with “device-detected SCAF” (which may include very short episodes, whose pathogenetic role is under debate) is currently lacking. Thus, a wait-and-see approach tend to be promoted in many clinicians, who prefer to continue surveillance for additional or longer episodes of AF instead of prescribing OAT [79]. In particular, in a retrospective study of 445 pacemaker patients from a single academic hospital there was a significantly lower usage of OAT in patients with subclinical pacemaker-detected AF than in patients with clinical AF (23.7% vs 58.9%; $p < 0.001$) [80]. In another report, OAT was administered in 61.3% of patients with device-detected AF, despite a mean CHADS2 score >2 [81]. In recently published trials about device-detected AF in patients with cryptogenic stroke (EMBRACE [31]; CRYSTAL-AF [32]; see also previous sections), increased detection led to significantly increased rates of anticoagulation use. In the EMBRACE trial, 13.6% of participants in the intervention group were switched from antiplatelet to anticoagulation, compared to 4.7% of the control group participants. As for the CRYSTRAL-AF trial, a significantly greater proportion of patients were prescribed OAT in the intervention group at 6 and 12 months (10.1 vs 4.6%, $p = 0.04$, and 14.7 vs. 6.0%, $p = 0.007$, respectively); by 12 months, 97% of the participants in the intervention group with AF were prescribed OAT. Of note, an electronic survey of practice in management of patients with silent AF among European Heart Rhythm Association Research Network partners from 33 centers in 16 countries revealed a strong consensus for OAT in patients with silent AF, including those with pacemaker-detected AF, with most responders considering device-detected AF as sufficient evidence to warrant anticoagulation [82]. On the other hand, IMPACT study has recently contributed to attenuate enthusiasm about anticoagulation, showing that introducing anticoagulation early in patients with implanted devices after detection of atrial tachyarrhythmia did not improve outcomes compared to standard of care [83].

Considering the complexity of the issue of diagnosis and treatment of patients with device-detected SCAF, further research is advisable [84]. To this purpose, Apixaban for the Reduction of Thrombo-Embolism in Patients with Device- Detected Sub-Clinical Atrial Fibrillation (ARTESiA) trial is worth to be mentioned. It is an ongoing, prospective, randomized (apixaban 5 mg twice daily versus aspirin 81 mg daily), double-blinded trial to determine whether anticoagulation reduces the risk of stroke and systemic embolism in patients with device-detected subclinical AF ≥ 6 minutes without a prior history of AF [85].

EMBOLIC STROKE OF UNDETERMINED SOURCE (ESUS): A NEW PRAGMATIC CLINICAL CONSTRUCT

Most non lacunar ischemic strokes are considered embolic in their origin (as hemodynamic mechanisms, vasospasm, and in-situ thrombotic occlusion are collectively less common causes than embolism) [86, 87]. Moreover, the fact that embolic obstructions typically recanalise spontaneously, resulting in open arteries resupplying the infarcted brain, is reported as a hallmark of embolic stroke [88-92]. On these premises, in 2014 Hart et al. [12] tried to overcome the “traditionally” vague, negatively defined entity of CIS with the more clinically useful, positively defined construct of “embolic stroke of undetermined source” (ESUS). Of course, ESUS stands outside the borders of “traditionally” well-defined cause of stroke (high-risk cardioembolic sources, large vessel atherosclerosis, lacunar stroke, rare diseases such as dissection and vasculitis). ESUS etiopathogenetic umbrella may include: minor-risk potential cardioembolic sources (structural abnormalities of mitral and aortic valve, atria and left ventricle; non-atrial fibrillation atrial dysrhythmias and stasis), covert paroxysmal atrial fibrillation, cancer-associated diseases, other arteriogenic emboli and paradoxical embolism.

ESUS was “positively” defined by the following diagnostic criteria [12]:

- Stroke detected by CT or MRI that is “not lacunar.” Lacunar stroke is defined as a subcortical infarct smaller than or equal to 1,5 cm ($\leq 2,0$ cm on MRI diffusion images) in largest dimension, including on MRI diffusion-weighted images, and in the distribution of the small, penetrating cerebral arteries; visualisation by CT usually needs delayed imaging greater than 24-48 hours after stroke onset.
- Absence of extracranial or intracranial atherosclerosis causing $\geq 50\%$ luminal stenosis in arteries supplying the area of ischemia
- No major-risk cardioembolic source of embolism (permanent or paroxysmal atrial fibrillation, sustained atrial flutter, intracardiac thrombus, prosthetic cardiac valve, atrial myxoma or other cardiac tumours, mitral stenosis, recent (<4 weeks) myocardial infarction, left ventricular ejection fraction less than 30%, valvular vegetations, or infective endocarditis.)
- No other specific cause of stroke identified (e.g., arteritis, dissection, migraine/vasospasm, drug misuse)

As for diagnostic assessment for ESUS, the following work-up was proposed [12]:

- Brain CT or MRI
- 12-lead electrocardiogram
- Precordial echocardiography
- Cardiac monitoring for ≥ 24 hours with automated rhythm detection (cardiac telemetry is not considered sufficient)
- Imaging of both the extracranial and intracranial arteries supplying the area of brain ischemia (catheter, MR, or CT angiography, or cervical duplex plus transcranial Doppler ultrasonography)

Of note, imaging of the proximal aortic arch is not needed; special blood tests for prothrombotic states are suggested only if the patient has a personal or family history of unusual thrombosis or associated systematic signs or disorder.

ESUS construct is supported by neuroimaging and pathological data. In a study of thrombi visualized with susceptibility-weighted imaging compared with thrombi assessed with digital subtraction angiography in patients with middle cerebral artery occlusion, the cause of stroke remained unknown in 31 of 88 patients (35%) [93]. In 30 of these patients (96%) with cryptogenic stroke, thrombotic material was visualized with susceptibility-weighted imaging without signs of underlying atherosclerotic disease shown by digital subtraction angiography, suggesting that embolic pathogenesis was more probable than in situ thrombosis.

Moreover, in a single-center series of 303 patients with ischemic stroke, Gratz et al. [94] reported about histopathological analysis of thrombotic material retrieved by endovascular means, finding hints of thromboembolism in the 79 patients (26,1%) with ESUS (ten had erythrocyte-rich thrombi, six had platelet-rich thrombi, and 63 had mixed thrombi).

As for prophylactic treatment of patients with ESUS, two international randomized trials (RE-SPECT-ESUS [95] and NAVIGATE ESUS [96]), comparing direct oral anticoagulants (dabigatran and rivaroxaban, respectively) with aspirin are currently ongoing.

CONCLUSION

CIS has long represented a negatively defined diagnostic and therapeutic area. Recent diagnostic advancements (such as loop recorder to detect SCAF) and new clinical construct (such as ESUS) are positively defined efforts to overcome CIS limitations. Moreover, many patients with CIS may benefit from anticoagulation; on top of that, recent and future drug development has the potential to maximize risk-benefit ratio and cost-effectiveness. In spite of exciting perspectives, several etiopathogenetic, diagnostic and therapeutic issues must be further elucidated, so that on-going trials and future research are warranted.

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Chapter 35

DIRECT ORAL ANTICOAGULANTS IN PATIENTS WITH ATRIAL FIBRILLATION

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ABSTRACT

Vitamin K antagonists' limitations are thought to be at least partially responsible for the underprescription of anticoagulants in a large percentage of eligible patients with atrial fibrillation. In the past few years, novel oral anticoagulants have been developed, directly blocking the activity of thrombin (dabigatran) or inhibiting activated factor X to produce anticoagulation (rivaroxaban, apixaban, edoxaban), thus also known as direct oral anticoagulants (DOACs). In patients with atrial fibrillation, DOACs have been reported to be at least equal to warfarin in reducing stroke risk and to have a better safety profile with generally fewer complications. Moreover, they do not need to be monitored by measurements of circulating levels or index of activity and are easy to manage in case of planned surgery or procedures. In this chapter we will describe findings from the recent Phase III trials investigating DOACs for stroke prevention in atrial fibrillation patients, with particular attention to topics of interest for clinical management by neurologists.

Keywords: DOACs, atrial fibrillation, ischemic stroke, cerebral hemorrhage

INTRODUCTION

Oral anticoagulation is crucial for the prevention of stroke in atrial fibrillation. Traditional vitamin K antagonists (VKA) reduce the risk of cerebrovascular events by more than 60% in patients with atrial fibrillation, but are also responsible for an increased

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hemorrhagic risk [1]. In addition, the need for INR monitoring prevents to get the best compliance to treatment. These limitations are probably responsible for the well-known underprescription of anticoagulants in patients with atrial fibrillation who should be treated according to guidelines [2]. A noteworthy number of patients discontinue warfarin treatment within one year from start [3]. In the past few years other oral anticoagulants have been developed: dabigatran, rivaroxaban, apixaban and edoxaban. They are known as novel, non-vitamin K dependent oral anticoagulants (NOACs) or direct oral anticoagulants (DOACs), because they directly block the activity of thrombin (dabigatran) or inhibit activated factor X (Xa) to produce anticoagulation (rivaroxaban, apixaban, edoxaban) without interfering with vitamin K-dependent coagulation factors. In patients with atrial fibrillation, DOACs have been reported to be at least equal to warfarin in reduce stroke risk and to have a better safety profile: all-cause mortality was significantly lower for DOACs compared to warfarin in large RCTs [4-7]. Moreover, they do not need to be monitored by measurements of circulating levels or index of activity. In this chapter we will discuss the most relevant features of DOACs and differences and advantages compared to traditional vitamin K antagonists. Finally, we will discuss in detail the topics of major interest from neurologists' perspective.

GENERAL OVERVIEW OF DIRECT ORAL ANTICOAGULANTS

Efficacy and Safety in Stroke Prevention

Four large randomised trials (RE-LY, ROCKET-AF, ARISTOTLE, ENGAGE-AF), including more than 72000 patients, have explored efficacy and safety of DOACs for stroke prevention in patients with atrial fibrillation eligible for oral anticoagulation with VKA [4-7]. As reported by RCT results and subsequent meta-analyses [8, 9], in atrial fibrillation patients DOACs were at least as effective as warfarin in preventing stroke (or significantly better for higher dose dabigatran and for apixaban). Also, a relative risk reduction in death of about 10% was observed. DOACs determined less serious bleeding than VKA [9]. In particular, intracranial bleeding rate was more than halved by comparison with VKA [10], although the absolute rate with warfarin is low (around 0.5% per year). In contrast, gastrointestinal bleeding with DOACs occurs 25% more often than with warfarin, with the only exception of apixaban [6]. These findings have been supported by observational analyses from registries [11, 12]. The safety advantage persists over time [13], and is particularly notable for intracranial hemorrhage.

The choice of which oral anticoagulant should be used in patients with atrial fibrillation is not mandatory according to current guidelines: the 2012 European Society of Cardiology guidelines support DOACs over VKA [14], while the 2014 American College of Cardiology / American Heart Association guidelines give DOACs and VKAs a similar level of recommendation [15]. Patients already treated with VKA should continue treatment if Time in Therapeutic Range (TTR) >60% in the last 6 months, unless concomitant bleeding conditions occur. Patients treated with VKA but with TTR ≤60% should be evaluated for a therapeutic shift to DOACs. Newly diagnosed atrial fibrillation patients should be treated with DOACs in case of practical difficulties in assessing INR values, concomitant other therapies with high risk of drug interactions, previous intracranial bleeding.

Practical considerations about the specific features of each anticoagulant could be used as guidance in targeting treatments according to individual patients' characteristics [16, 17]. For example, patients older than 80 years should be treated with dabigatran 110 mg bid instead of 150 mg bid, since the higher dosing has been associated with excess bleeding in the elderly. Patients with gastrointestinal vulnerability or previous bleedings should be treated with apixaban, since it was the only DOAC with a lower rate of GI bleedings compared to warfarin [6]. Patients with high stroke risk without significant bleeding risk could benefit from dabigatran 150 mg bid, which produced the largest reduction in risk of ischemic stroke [4], rivaroxaban or edoxaban, since they were tested on a population with a large percentage of patients with CHADS₂ score ≥ 3 (87 and 53%, respectively) [5, 7]. In patients with high stroke risk but also high hemorrhagic risk, dabigatran 110 mg bid, apixaban or edoxaban were reported to be significantly safer than warfarin [4, 6, 18].

In atrial fibrillation patients with contraindications to VKA, therapeutic options could include dual antiplatelet treatment or apixaban, as reported by the results of the AVERROES trial: in nearly 5600 VKA-unsuitable patients, apixaban reduced stroke or systemic embolism by 55% compared with aspirin with a similar risk of major bleeding (1.4%/year with apixaban versus 1.2%/year with aspirin) including intracranial hemorrhage [19].

Thanking to their efficacy, safety, ease of administration without need of anticoagulation monitoring, DOACs are expected to replace VKA in most patients. It is important to note that DOACs are more expensive than VKA. However, the extra cost for health-care systems appears to be justified by cost-effectiveness analyses [15, 20]. To date, there are no studies directly comparing the different DOACs between them.

Pharmacological Aspects

DOACs have quite predictable pharmacological effects: a fast onset of activity (2–3 h to peak effect) and a relatively short duration of action, ensuring rapid recovery of hemostatic ability in case of bleeding or planned surgery. Moreover, bridging with heparins is not required. Despite similar half-lives of about 12 h for all four drugs, dabigatran and apixaban were developed with twice per day dosing versus once per day dosing (for atrial fibrillation) with rivaroxaban and edoxaban.

Unlike VKA, DOACs have relatively few drug and food interactions; therefore anticoagulation monitoring is not required. Rivaroxaban has 40% more gastrointestinal absorption when taken with a high calorie meal, and for this reason it is generally recommended to take it with the most consistent meal of the day. Since patients with atrial fibrillation are usually treated with many drugs, when prescribing DOACs it is important to bear in mind the possibility of interactions, especially with CYP3A4 and P-glycoprotein inhibitors. However, drug interactions with DOACs are fewer than with VKA [21–25].

Contraindications

When prescribing DOACs, absolute contraindications to anticoagulants have to be considered: pregnancy, known hypersensitivity, severe ongoing hemorrhage and conditions of high bleeding risk such as hemorrhagic diathesis and platelet count $<30000/\mu\text{l}$, hepatic or

renal failure. Relative contraindications include mild to moderate ongoing hemorrhage or ulcer affecting GI tract; intracranial hemorrhage; intra or extra cranial aneurysms; pericarditis; bacterial endocarditis; previous intracranial/ endocular/ spinal/ retroperitoneal hemorrhage.

Moreover, also the exclusion criteria from the registration studies [4-7] must be kept in mind when prescribing DOACs: reduced renal function (GFR <30 ml/min for dabigatran and rivaroxaban, <25 for apixaban); uncontrolled hypertension; current hepatopathies; platelet count <100000/ μ l; concomitant treatment with acetylsalicylic acid (ASA, >100 mg die for rivaroxaban and >165 mg die for apixaban) or with ASA plus clopidogrel (apixaban); recent treatment with ASA plus thienopyridines in the last 5 days (rivaroxaban); recent treatment with intravenous antiplatelets or fibrinolytics (in the last 48 hours for dabigatran, in the last 5 days for rivaroxaban) [4, 5]. In addition, DOACs should not be prescribed in patients with atrial fibrillation and significant valvular disorder.

Concomitant therapies should always be considered to avoid drug interactions.

Adherence Issues

VKA efficacy is considered to be strictly depending on patients' compliance. However, besides the advantages in management, also DOACs can be problematic in case of poor adherence because of their short half-life. Especially when DOACs are administered once a day, one missed dose results in low trough drug concentrations. Moreover, a routine measurement of the effect of the drug is not currently available for DOACs, making more difficult to assess the quality of patients' compliance [26]. However, adherence to DOACs in atrial fibrillation patients does not seem to be different from that of warfarin (75% in the case of dabigatran) [9, 27, 28].

Some problems in clinical management could arise from suboptimal adherence. In the absence of conclusive data from RCTs, practical guidelines have been proposed by experts [18]. For example, a missing dose could be made up within 6 hours for drugs that are usually taken every 12 hours and within 12 hours for drugs usually taken every 24 hours. After these timings, the dose must be "skipped." If the dose has been erroneously taken twice, it is suggested to skip the following dose for drugs usually taken every 12 hours and instead to keep the following habitual dose for drugs usually taken every 24 hours. If it is not possible to ensure the effective ingestion of the last dose, for drugs usually taken every 12 hours no additional dose is required, while for drugs usually administered every 24 hours it is advised to take another dose.

PRESENCE OF COMORBIDITIES

Chronic Kidney Disease

DOACs have renal clearance, especially dabigatran (80%). In fact, the assessment of creatinine clearance is fundamental to choose and determine dosing of these drugs. DOACs have not been clinically studied in patients with stage IV chronic kidney disease (creatinine

clearance <30 mL/min), therefore are contraindicated in such patients; moreover, the inefficient drug elimination by kidneys can result in drug accumulation, and thus in increasing hemorrhagic risks. Patients with milder chronic kidney disease (creatinine clearance 30-50 mL/min) could be treated with reduced doses of dabigatran (110 mg bid), rivaroxaban (15 mg) and edoxaban (30 mg); in patients with creatinine ≥ 1.5 mg/dL, dosing of apixaban should be reduced to 2.5 mg bid only in case of concomitant age ≥ 80 years and/or weight ≤ 60 kg. Rivaroxaban and apixaban should be preferred considering the lower rate of renal excretion [26]. In addition, regular kidney function checks should be performed especially in elderly patients.

Acute Coronary Syndromes

20-40% of patients with atrial fibrillation have concomitant coronary artery disease [29]. The combination of oral anticoagulants and antiplatelet therapy for patients with an indication for both is challenging, mainly because of the increased bleeding risk [30]. Since VKA are effective in preventing coronary thrombotic events, current European guidelines for atrial fibrillation management discourage the use of antiplatelet therapy for patients with stable coronary disease treated with oral anticoagulants [14]. In patients undergoing percutaneous coronary intervention, especially in the setting of acute coronary syndromes, during and after the procedure potent antiplatelet therapy is needed to prevent thrombotic complications including stent thrombosis. In such cases dual antiplatelet therapy (aspirin plus clopidogrel) is added to VKA for at least one year after the intervention with any type of stent used [31, 32].

Thanking to their proven safety, using DOACs instead of VKA could be an option to reduce the risk of bleeding. At least two RCTs have been initiated and are currently ongoing to evaluate the safety and efficacy of a DOAC versus VKA in atrial fibrillation patients undergoing coronary intervention for either stable coronary disease or acute coronary syndrome [26]. European Society of Cardiology - European Heart Rhythm Association (EHRA) guidelines state that patients with atrial fibrillation and acute or stable coronary artery disease who require angioplasty and/or stenting, can be treated with a DOAC plus single or dual antiplatelet according to their bleeding risk and the clinical setting [33].

Elderly Patients

Elderly patients have been considerably studied in the registration studies for DOACs: 7258 in the RE-LY (4828 of which were treated with dabigatran), 6164 in the ROCKET (3082 receiving rivaroxaban), and 5678 in the ARISTOTLE (2850 treated with apixaban) [4-6].

Older age is a strong risk factor for both ischemic and hemorrhagic stroke. Older patients treated with dabigatran had a relatively greater risk of bleeding versus warfarin and compared with younger patients [34]. Accordingly, the European Society of Cardiology recommends the lower dose of dabigatran (110 mg bid) for patients aged ≥ 80 years [14]. Oral Xa inhibitors had a relative risk of bleeding versus warfarin quite similar in elderly patients compared with younger patients [35, 36].

The risk of overdosage of DOACs in the elderly is increased owing to the common renal impairment, low body weight and drug interactions [37]. A periodic monitoring of renal function by means of creatinine clearance assessment is therefore recommended.

Adherence to DOACs is of major importance due to their short half-lives. Elderly patients could have more adherence issues than younger patients; therefore the introduction of these treatments should be carefully discussed also with caregivers.

Reversal Strategies and Management of Bleedings

DOACs are associated with lower, but not absent, occurrence of bleedings. In case of serious hemorrhage, assessing anticoagulation status might be crucial. Since DOACs half-lives are about 12 h, to determine the timing of the last ingested dose allows estimating the anticoagulation status. To date, few accurate tests are commonly available. Dabigatran influences the activated partial thromboplastin time (aPTT): a normal aPTT suggests relatively little dabigatran effect. Also, dabigatran plasma drug level is directly correlated with anticoagulation effect [38]. Rivaroxaban usually produces at least slight elevation of the prothrombin time (PT). A modified thrombin time and anti-factor Xa assays are commercially available to establish the effect of dabigatran and direct Xa inhibitors, respectively; however, these assays are neither widely nor rapidly available in the emergency settings. Creatinine level should be measured to assess renal function and estimate the rate of anticoagulant drug clearance.

In case of serious bleeding, patients should be hospitalized. Clinical management is based on: stop of any antithrombotic therapy, identification of the source and local strategies to produce hemostasis. Within 2–4 h from DOAC ingestion, oral activated charcoal will reduce absorption. Fluid resuscitation and blood transfusions might be indicated according to blood panel. Reversal of other antithrombotics should be considered (for example platelet transfusion if ongoing treatment with aspirin). If treated with dabigatran, patients could benefit from hemodialysis (particularly if aPTT >80 sec), frozen fresh plasma or desmopressin acetate. Restoration of coagulation could be partly achieved by the administration of prothrombin complex concentrate [39] or activated factor VII, although no clinical evidence or practice guidelines are currently available. Similar strategies can be applied in case of need of urgent, not extensible surgery.

In patients treated with VKA a reversal strategy with vitamin K and coagulation factors is well established, although not always associated with better outcome owing to delayed efficacy. In contrast, antidotes for DOACs are not yet routinely available, although reversal agents have been currently developed and are currently studied in clinical settings [40–42]: idarucizumab is a humanized monoclonal antibody against dabigatran, which has been recently approved by FDA [43]; andexanet is a decoy (truncated) factor Xa molecule that binds Xa blockers; finally, PER977 binds non-specifically all DOACs [26, 44].

Although relatively uncommon by comparison with VKA, intracranial bleeding occurs also in patients on DOACs. Intracranial hemorrhage in association with any oral anticoagulant has a poor prognosis, independently on reversal strategies whose impact on clinical outcome is unclear. According to this perspective, the use of DOACs could be supported because it is associated with lower rate of intracranial hemorrhage compared to VKA.

Gastrointestinal bleedings are instead more common during DOACs (with the only exception of apixaban) than VKA therapy. Patients with medical history of gastrointestinal bleeding have to be carefully examined if having indication to DOACs. Concomitant treatment with proton-pump inhibitors could be considered, although there is no evidence supporting this strategy. Concomitant treatment with non-steroidal anti-inflammatory drugs and antiplatelets, which is associated with higher rate of major bleeding [30, 45], should be avoided, unless clearly indicated.

Switching between Anticoagulants

When switching an eligible patient from VKA to a DOAC, the gap in therapeutic anticoagulation status must be the shortest possible to avoid thrombotic events [46]. After stopping VKA, when INR has dropped to around 2.0 or less, the DOAC should be initiated. Bridging with parenteral anticoagulation is not generally advised.

When switching from a DOAC to VKA is required (for example for deteriorating renal function), due to the slow onset of action of VKA, the DOAC and VKA should be given concomitantly until the INR is around 2.0. Since oral direct factor Xa blockers affect INR early after intake, INR should be checked immediately before the next DOAC intake during the concomitant administration period, and retested 24 h after the last DOAC dose to ensure that the patient is in therapeutic range on the VKA. After the transition is completed frequent INR checks should be made, as in any other patient being initiated on a VKA.

For patients treated with LMW heparin, start the DOAC when the next LMW heparin is due. For conversion from a continuous heparin infusion, DOAC should be started immediately when infusion ceased. To convert from DOAC to unfractionated or LMW heparin, these have to be started 12-24 hours after the last DOAC dose (no bolus dose of unfractionated heparin is required). It is better to wait 48 hours if ongoing treatment with dabigatran and creatinine clearance < 30 ml/min.

Management in Case of Planned Cardioversion

Among DOACs, only rivaroxaban has completed a Phase III clinical trial with the specific indication for anticoagulation of patients undergoing electrical or pharmacological cardioversion, resulting to be effective and safe as warfarin [47]. Practical recommendations from EHRA suggest that cardioversion is acceptably safe in patients treated with any DOAC: in fact, in the registration studies the subgroups of patients undergoing cardioversion treated with DOACs always showed lower rates of complications compared to warfarin [18]. DOAC treatment should be initiated 3 weeks before the procedure, and continued at least for other 4 weeks after. In case of uncertain compliance, transesophageal echocardiography should be performed before cardioversion to ensure the absence of intracardiac thrombosis.

Management in Case of Planned Surgical Interventions

Thanking to their short half-lives, DOACs are of easy management in case of planned surgery. It can be hypothesized that a brief suspension of treatment would result in reversal of the anticoagulant effect without need of bridging with heparins, in contrast with VKA.

However, few data from clinical experience in such settings are currently available. To date, the only recommendations come from experts [18]. Generally, in such conditions it is important to consider: patients' features (age, renal function, ongoing treatments), drug features and hemorrhagic risk related to the procedure.

Low bleeding risk procedures include: endoscopic procedures with bioptic sampling, prostatic or bladder biopsy, pacemaker implantation, electrophysiological procedures, radiofrequencies ablation, angiography. In patients with normal kidney function, procedures should be planned at least 24 hours after the last DOAC administration. In patients with reduced renal function, the procedure could be performed at least 36 and 48 hours after last dose of dabigatran if GFR 50-80 and <50 ml/min, respectively, and 36 hours after last dose of apixaban or rivaroxaban if GFR 15-30 ml/min. A very low hemorrhagic risk is usually hypothesized in cases of odontoiatric interventions and dental avulsion, surgical corrections of cataract and glaucoma, minor dermatological interventions (especially if effective local hemostatic measures could be applied), and also endoscopic procedures without bioptic sampling. In these cases DOAC treatment should not be suspended especially if the intervention could be planned during the end of the therapeutic window of the drug (12 hours after last dose for dabigatran and apixaban and 24 hours after last dose of rivaroxaban). Subsequently, patients should be carefully observed to ensure efficacy of local hemostatic measures. After low and very low risk procedures, DOACs can be restarted after 6-8 hours [18].

Procedures with high hemorrhagic risk include: lumbar puncture, epidural anesthesia, neurosurgery, esophageal varices ligation, endoscopic removal of polypi, sphincterotomy and dilation of stenosis, thoracic and abdominal surgery, orthopedics, hepatic or renal biopsy, transurethral resection of prostate. In those cases, treatment with DOACs should be managed under strict surveillance of expert specialists in coagulative disorders. To get a minimal residual anticoagulant effect (<3-6%) at the time of surgery, it has to be planned to stop DOAC ingestion 4 or 5 drug half-lives before [48]. Bleeding risk can also be minimized adjusting the time when anticoagulant is resumed. For patients at high risk for thromboembolism, it is possible to restart with a reduced dose of dabigatran or rivaroxaban 12-24 hours after the procedure, and resume the full dosage only after 48-72 hours. Patients with low ischemic risk could wait 72 hours or more after surgery [48]. Patients with subsequent need of immobilization should be treated with prophylaxis for deep venous thrombosis (LMW heparin) 6-8 hours after the intervention and then restarting DOAC after 48-72 hours.

DOACs FROM NEUROLOGIST'S PERSPECTIVE

DOACs usually come to neurologists' attention in some specific clinical scenarios. In this section we will describe the more common clinical settings where DOACs have to be prescribed or managed by neurologists.

Secondary Prevention of Ischemic Stroke in Patients with Atrial Fibrillation

The CHADS₂ or the newer CHA₂DS₂-VASc scores are useful clinical tools in determining the appropriateness of initiating anticoagulation therapy in patients with atrial fibrillation. In both scoring system two points are assigned for medical history of TIA or stroke, and a final score of 2 or greater is considered high risk and gives recommendation for oral anticoagulation. Therefore, atrial fibrillation patients suffering from a cerebrovascular event should invariably deserve oral anticoagulants if not contraindicated.

Considering the study features of the main RCTs, patients with a medical history of one or more previous cerebrovascular events should benefit more and therefore be treated with apixaban, which was associated with the largest reduction of risk compared to warfarin [6], or rivaroxaban, since more than half (55%) of the patients tested in the ROCKET-AF trial experienced previous strokes [5]. Dabigatran at the dose of 150 mg bid was actually shown to be superior to warfarin for stroke prevention, but only nearly 20% of the studied population suffered from previous cerebrovascular events [4].

Adherence Issues in the Elderly and in Patients with Cognitive Impairment

As we previously outlined, keeping adherence to DOACs is crucial due to their short half-lives. Neurologists have often to manage patients with older age and possibly cognitive impairment. In particular, adherence to oral anticoagulation seems to be hampered mostly by reduced levels of physical and functional abilities (assessed by ADL and IADL scores), rather than by the presence of cognitive impairment itself (MoCA score <26) [49]. To ensure the best adherence to treatment, before prescribing anticoagulants neurologists should carefully focus on cognitive and mental status, gait performances and global disability, to assess what kind of supervision may be necessary for the patients, and discuss the features of the treatment with caregivers.

The Acute Phase of Stroke

The use of anticoagulants in acute stroke patients has been proposed to avoid neurological deterioration, to prevent cardioembolic recurrences and to improve outcome. Acute treatment with unfractionated heparin, LMW heparin or heparinoids for the acute phase of stroke showed in some studies a reduced rate of early ischemic events, but the overall clinical benefit was abolished due to increased hemorrhagic events [50-52]. Hemorrhagic transformation is a common complication after cardioembolic stroke, although is often asymptomatic. However, it is also true that anticoagulants increase risk of intra- and extracranial hemorrhage by 2%. Therefore, it would be safe stratifying patients according to their bleeding risk (considering stroke volume, expected spontaneous or thrombolysis-induced reperfusion timing, arterial pressure) to avoid early anticoagulation especially in high-risk cases, for which it is usual practice to wait about two weeks before starting anticoagulants. In any case, the use of anticoagulants in the acute phase of stroke is not supported by evidence (the fewer recurrent ischemic strokes were offset by an increase in extracranial bleedings) [53] and thus not recommended by current guidelines, which instead

suggest the use of aspirin within 24-48 hours from stroke onset [54]. However, a recent study found that the best timing to start anticoagulation in secondary prevention of stroke is between 4 and 14 days from stroke onset: this timing was associated with a lower rate of a composite outcome measure considering cerebral and systemic embolism and cerebral and systemic bleeding [55]. Although DOACs have been associated with fewer hemorrhagic complications than warfarin, it is important to point out that the registration studies explicitly excluded patients in the acute phase of stroke (within 14 days for dabigatran and rivaroxaban, within 7 days for apixaban and within 30 days for edoxaban), therefore no data about safety and efficacy in such conditions can be deduced. Recently, some ongoing trials are exploring risks and benefits of acute treatment with DOACs [56, 57].

Moreover, it is important to note that also recent treatment with thrombolysis was considered an exclusion criterion in some of the DOACs registration studies (performed in the last 5 days for rivaroxaban, in the last 48 hours for dabigatran), therefore no data about safety of starting treatment with DOAC in patients with acute stroke undergoing thrombolysis are currently available.

Ischemic Stroke Recurrences during Treatment with DOACs

Unfortunately, also patients treated with DOACs could suffer from acute ischemic stroke recurrences.

Acute Intervention Strategies

An ongoing effective treatment with anticoagulants would contraindicate thrombolytic therapies. With VKA, testing INR allows to directly assess the anticoagulation status. With $\text{INR} \leq 1.7$, intravenous thrombolysis can be performed [54, 58]; if not, the only option to consider is an endovascular approach. Management of ischemic recurrences during treatment with DOACs is more challenging due to the absence of a reliable anticoagulation index. Therefore, to determine the timing of the last ingested dose becomes crucial. If it can be ensured that the last dose was taken at least 48 hours before, and laboratory tests give normal values of INR, PT and aPTT, there would be enough confidence to treat the patient safely. If it is not possible to determine the last DOAC administration, some laboratory values could help the decision-making process. It is useful to remember that the aPTT is moderately increased by dabigatran, but not by Xa-inhibitors, which instead modify PT and INR (only at their peak concentration). Unfortunately, the more specific and reliable tests assessing DOACs' activity are not commonly available in hospitals and emergency settings. They are thrombin time (TT, and the more appropriate diluted thrombin time – dTT or Hemoclot), ecarin clotting time (ECT), both notably increased during treatment with dabigatran, and anti-factor Xa activity (increased by direct Xa-inhibitors as well as by LMW heparin) [59]. If it is not possible to determine the timing of last DOAC ingestion but common laboratory tests (aPTT, INR) are normal, the suspect of an inadequate anticoagulation can only be partially supported, and thus it would be better to carefully discuss with patients and caregivers the risks of an eventual intravenous thrombolysis. Or, if neuroimaging gives evidence of a proximal arterial occlusion, endovascular procedures could be considered. If thrombin time

and ecarin clotting time are available, their normality after 4 hours from the last dabigatran ingestion could exclude a clinically relevant anticoagulation. Similarly, anti-factor Xa activity after 5 hours from the last Xa-inhibitor rules out its effect [60, 61]. Thus, emergency treatment with alteplase could be applied. Within at least 12 hours from last dabigatran dose and $TT < 4$ times the upper limit of the normal range and/or $ECT < 2$ times the upper limit of the normal range, intravenous thrombolysis could be proposed, carefully evaluating pros and cons, as well as intrarterial approaches [59].

Implementation of Secondary Prevention Strategies after their Failure in Preventing Recurrences

As already mentioned, Phase III trials did not explore the effect of DOACs in the acute phase of stroke [4-7]. Therefore, after the occurrence of an ischemic event during treatment with DOACs, restarting anticoagulation can be challenging. EHRA practical guidelines suggest that continuation of DOAC treatment should be made according to the stroke volume. It is suggested to restart DOAC one day after a TIA, three days after a small, no disabling stroke, six days after a moderate stroke and at least 12 days after a large stroke (the so-called “1-3-6-12” rule) [18]. Ischemic recurrences occurring during adequate DOAC treatment do not allow increasing the dosage, in contrast with the traditional, quite common practice of increasing target INR with VKA. A good practice would be to carefully examine the etiology of the stroke recurrence, assess comorbidities and recalculate the ischemic risk of the patients. An acute lacunar syndrome should lead to implement pharmacological control of the more common risk factors: hypertension and diabetes. In the presence of some comorbidities (ischemic cardiopathy, severe atherosclerosis), the addition of an antiplatelet could be considered. However, particular attention should be made, since Phase III trials considered as contraindications to randomization a concomitant treatment with acetylsalicylic acid (ASA, >100 mg die for rivaroxaban and >165 mg die for apixaban) or with ASA plus clopidogrel (apixaban) and recent treatment with ASA plus thienopyridines in the last 5 days (rivaroxaban) [5, 6]. Still, the clinician must consider the higher bleeding risk associated with these changes, and this must be communicated to the patient.

Intracranial Hemorrhage

Intracranial hemorrhage is the most devastating complication of oral anticoagulation, determining high mortality and disability in survivors. Although infrequent, intracranial bleeding can occurs also in patients treated with DOACs. It has been reported that a cerebral hematoma arising during dabigatran treatment tends to remain small without significant expansion [62]. However, intracranial bleeding associated with any antithrombotic treatment is often affected by a poor prognosis.

The absence of approved specific reversal strategies further affect clinical management of this condition. Hospitalization and general measures for acute bleeding have to be urgently applied (see above) and laboratory tests performed in order to plan eventual strategies to anticoagulation reversal and fluid or blood transfusions. However, the impact of anticoagulation reversal on clinical outcome has not been sufficiently explored. To date, the

best clinical practice against bleeding is therefore based on its prevention. DOACs could be preferred over VKA due to the lower incidence of intracranial hemorrhage. Then, it is important to carefully evaluate the risk profile of the patients that could receive antithrombotics before prescription.

Clinical and Neuroimaging Tools to Stratify Hemorrhagic Risk

To date, there are a few clinical tools that may guide clinicians in assessing bleeding risk. The HAS-BLED scoring system has been recently incorporated into the European Society of Cardiology guidelines for management of atrial fibrillation, although it requires further validation. This scoring system assigns 1 point for each of the following risk factors: hypertension, abnormal renal function, abnormal liver function, medical history of stroke, previous significant bleeding, labile INR, age older than 75, chronic use of aspirin/ NSAIDs, chronic use of drugs or alcohol. A score ≥ 3 or greater than the CHADS₂ score, it is assumed that bleeding risk outweighs the potential benefit of oral anticoagulants [63, 64]. Besides HAS-BLED items, congenital or acquired coagulative disorders, ulcerative GI disease are other not uncommon conditions associated with an increased hemorrhagic risk. If treated with anticoagulants, these patients have to be routinely checked with periodic blood count and in search also for minor bleeding (for example, occult bleeding in stools or urine).

Besides risk scores, recently brain imaging (in particular, MRI) has been proposed as another clinical tool to assess potential complications and predict outcome of anticoagulant treatment. Cerebral microbleeds are defined as small, round areas of hypointensity on the gradient echo T2-weighted magnetic resonance sequences, and are believed to represent perivascular accumulation of hemosiderin-containing macrophages, suggesting vulnerability of the cerebral small vessels. Cortical microbleeds are currently thought to be manifestations of cerebral amyloid angiopathy, while deep hemisphere lesions would be indicative of hypertensive arteriopathy. They have been hypothesized to be a biomarker for bleeding-prone small vessel pathology, and thus could predict future risk of developing symptomatic intracerebral bleeding. Cerebral microbleeds have been described in association with other conditions expressing vulnerability of cerebral small vessels: older age, hypertension, smoke, lacunar infarcts, prior ischemic strokes or intracranial hemorrhages, white matter disease, and Alzheimer disease. Some of these conditions overlap with CHA₂DS₂-VASc items, indicating that this pro-hemorrhagic cerebral condition can be likely associated with a high ischemic risk. White matter changes are another condition that can affect cerebral ageing to various extents, the more severe known as leukoaraiosis, and have been shown to be strongly related to presence of cerebral microbleeds [65], suggesting a common microvascular etiology for both. However, the predictive value of white matter disease detected by MRI for anticoagulant-related intracranial hemorrhage is not currently known [66].

Table 1. Summary of DOACs features

DOAC name	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Trade name	Pradaxa	Xarelto	Eliquis	Savaysa, Lixiana
RCT Registration study	RE-LY	ROCKET-AF	ARISTOTLE	ENGAGE-AF
Design	Open label	Blinded	Blinded	Blinded
Mean CHADS ₂ score	2.1	3.5	2.1	2.8
Target	Factor IIa	Factor Xa	Factor Xa	Factor Xa
Daily dosing	2	1	2	1
Renal clearance	≈80%	≈35%	≈25%	≈50%
Elimination by means of hemodialysis	Yes (68%)	Partial	No	No
Biodisponibility	3-7% (keep intact capsules to avoid increased Biodisponibility)	66% fasting, 100% after meal	50-60%	62%
Effect of food on drug absorption	None	Increased	None	None
Effect of PPI on drug absorption	Reduced (12-30%)	None	None	None
Time to peak concentration	1.25-3 h	2-4 h	3-4 h	1-2 h
Half-life	12-17 h	5-9 h (11-13 h if age ≥75 y)	8-15 h	8-10 h
Time of anticoagulant effect reversal	24 h	24 h	24 h	24 h
Dose	150 mg bid / 110 mg bid (if age ≥80 y, creatinine clearance 30-50 ml/min, high bleeding risk or concomitant therapy with verapamil)	20 mg / 15 mg (if creatinine clearance 30-50 ml/min)	5 mg bid / 2.5 mg bid (if 2 of the following criteria are met: age ≥80 y, weight ≤60 kg, creatinine ≥1.5 mg/dl, or if creatinine clearance 15-29 ml/min)	60 mg / 30 mg (if creatinine clearance 30-50 ml/min, weight ≤60 kg or strong P-glycoprotein inhibitor)
Metabolism	P-glycoprotein	P-glycoprotein and CYP3A4	P-glycoprotein and CYP3A4	P-glycoprotein
Drug interactions	Atorvastatin, PPI, NSAIDs, ketoconazole, dronedarone, rifampicin	Ketoconazole, itraconazole, voriconazole, posaconazole, ritonavir, rifampicin, azithromycin, phenytoin, carbamazepine, phenobarbital, diltiazem, verapamil, hypericum	Diltiazem, verapamil, azithromycin, hypericum	Ketoconazole, quinidine, erythromycin, amiodarone
Food interactions	None*	None*	None*	None*
Binding to plasma proteins	35%	85%	90%	50%

Table 1. (Continued)

DOAC name	Dabigatran	Rivaroxaban	Apixaban	Edoxaban
Preferred laboratory test / Emergency scenario test	Dilute thrombin time / Accelerated partial thromboplastin time	Anti Xa-concentration / Prothrombin time	Anti Xa-concentration / Diluted prothrombin time	Anti Xa concentration /No data available
Antidote	Idarucizumab PER977	Andexanet alfa PER977	Andexanet alfa PER977	Andexanet alfa PER977

* With the exception of grapefruit juice, which is a well-known strong P-gp inhibitor.

A brain MRI has been proposed as an additional tool to stratify hemorrhagic risk. The presence of cortical microbleeds or at least five subcortical microbleeds would discourage traditional vitamin K-dependent anticoagulation, while novel anticoagulants would be acceptable. The development of any neurological changes during treatment with anticoagulants would indicate to perform a follow-up MRI to investigate potential microbleed progression. Due to the high costs of the technique, MRI screening could be applied in some particular settings to help the decision-making process, for example in patients with intermediate CHADS₂ scores, or in patients whose fall risks or cognitive impairment could increase bleeding risk [66].

Restarting DOACs after Intracerebral Hemorrhage

After an anticoagulant-associated intracerebral hemorrhage, the resumption of anticoagulation has to be carefully evaluated, by considering thromboembolic and hemorrhagic risks, the treatment history with anticoagulation, the causes of the hemorrhages, the comorbidities of the patient, the risk of hematoma growth. Patients with typical, deep hemispheric intracerebral hemorrhage and a CHA₂DS₂-VASc score ≥5, may benefit from restarting anticoagulation, which basing on the available data would reasonably be after 10 weeks although some works have suggested an earlier resumption [67, 68]. A careful assessment of other pro-hemorrhagic conditions, for example uncontrolled hypertension, should be done and risk factors addressed with adequate treatments [69]. In patients with atypical (lobar) cerebral hemorrhage or cerebral amyloid angiopathy, the risk for anticoagulant-related hemorrhagic recurrences is higher than the thromboembolic risk and for this reason anticoagulation should be avoided [67]. Non-pharmacological prevention strategies of thromboembolism, such as surgical ablation or left atrial appendage occlusion, should be evaluated as alternatives to anticoagulants [70, 71].

CONCLUSION

A large proportion of patients with atrial fibrillation and risk of stroke, although eligible for anticoagulation, are treated suboptimally. The oral direct inhibitors of factors IIa or Xa provide an important alternative to traditional VKA, being at least as effective as warfarin for stroke prevention in atrial fibrillation with a more favorable safety profile, especially in terms

of reduced intracranial bleeding, and potentially easier adherence. To date, the difficult reversal of DOACs effects has not significantly worsened bleeding outcomes. However, the safe management of these drugs requires experience and knowledge about their pharmacological properties, contraindications and specific clinical indications.

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Chapter 36

EMERGING BIOCHEMICAL RISK MARKERS OF STROKE

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ABSTRACT

Stroke is a multifactorial event, resulting from the combination of genetic and environmental determinants. The first are more influent in younger patients, while the importance of the environmental factors increase with the age of patients. Biomarkers are thus needed, especially in older persons, to define the current risk, as it results from the individual combination of genetic predisposition, environmental exposure, and clinical history.

The presence of atherosclerosis is a major cause of stroke. The well established risk factors for atherosclerosis (e.g., dyslipidaemia, hypertension, diabetes, obesity) allow the identification of patients at higher risk of vascular events, nevertheless, a large proportion of events still occur, even in patients apparently at low risk. Thus new biomarkers are needed to better identify high-risk patients, and to achieve further insights into the pathogenesis of stroke and its causative conditions.

In the first part of this chapter, inflammation, thrombosis, oxidative stress are discussed as possible source of biomarkers of plaque progression and destabilization. In the second part, serum gamma-glutamyltransferase is presented as a novel marker for cardiovascular prognosis related to atherosclerosis.

Keywords: stroke, biomarkers, atherosclerosis, gamma-glutamyltransferase

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INTRODUCTION

Stroke is a multifactorial event, resulting from the combination of genetic and environmental determinants. Genetic factors have been found to contribute to the risk of stroke in several ways, and at multiple levels of its pathogenesis, e.g., by increasing susceptibility to conventional risk factors, by enhancing the potency of their effects, or simply as additive independent pathogenic factor [1]. In younger patients, extensive molecular genetic studies, and in particular the adoption of Genome-Wide Association Study approach, will help identifying new genes contributing to the risk of stroke, and improving several aspect of the clinical management of the disease. Nevertheless, it is quite obvious that especially in older patients the influence of the environment on the risk of stroke is proportionally larger; cardiovascular disease, and atherosclerosis in particular, are a major cause of stroke, and the individual atherosclerotic burden, and consequently the risk profile, largely results from the combination of genetic predisposition and of the severity/duration of environmental influences, such as individual behaviour, lifestyle, professional exposure, previous or current disease etc., that may contribute to the onset and progression of the conditions at the basis of stroke. Biomarkers are thus needed, especially in older individuals, to define the current risk, as it results from the individual combination of genetic predisposition, environmental exposure, and clinical history.

In this perspective, the understand of the role of cholesterol in the pathogenesis of the atherosclerotic plaque, the elucidation of the biochemical and molecular aspects of cholesterol synthesis, the development of a suitable clinical interpretation of serum cholesterol levels, the development of inhibitors of the endogenous cholesterol synthesis, have been an extraordinary achievement of modern medicine, and an example of fruitful interaction between basic and clinical research, which already allowed to interfere directly with evolution of extracranial and intracranial atherosclerosis and related events, and had a recognized effect in reducing the individual risk of stroke. Nevertheless, a large proportion of events still occur, even in patients apparently at low risk, or despite prevention, thus making urgent the identification of new biomarkers that may allow the identification of high-risk population deserving a more aggressive intervention, but also to be used as tools to achieve further insights into the pathogenesis of stroke and its causative conditions.

THE PATHOLOGICAL BASIS OF ARTERIAL WALL INFLAMMATION

The clinical sign of atherosclerosis is the formation of the fibro-lipid plaque (atheroma) in the wall of the large- and medium-size arteries. These lesions are extremely common in adults, and their natural history proceeds very slowly, spanning all over the entire life. The initial phase of the lesion is represented by endothelial damage that favours the accumulation of oxidized lipids and cellular infiltration of the intima; this lesion can evolve into a fibro-lipid plaque. This second phase is characterized by the simultaneous formation of a core, where oxidized lipids and cellular elements of phagocytic nature accumulate, and of a fibrous cap that serves as a container for the inflammatory tissue [2]. Over the decades, the progressive growth of the lesion, due both to the accumulation of material and to the proliferation of reactive cellular elements, can determine its protrusion in the vascular lumen

with progressive reduction of blood perfusion of tissue placed downstream of the affected vessel. This phenomenon may have morphological (atrophy and remodelling) as well as functional (failure) consequences on the affected tissue. The most dangerous and frequent consequences of atherosclerosis consist in acute events such as myocardial infarction and stroke, due to the sudden obstruction of blood flow in the vessel affected by the atheroma, caused by thrombotic or intra-plaque haemorrhagic events, largely independent of the size of the lesion [3-6].

Most of the individuals carrying plaques will never develop clinical consequences of atherosclerosis, and, for this reason, in the follow-up of patients with evidence of atherosclerosis a particular attention is devoted to the identification of those at a high risk of developing acute events, deserving a more intensive intervention [7-10].

The occurrence of the individual clinical consequences of atherosclerosis depends on the specific structural evolution of the lesions, thus on biochemical and molecular events occurring within the plaques. In the case of chronic consequences, proliferative and hyperplastic aspects prevail causing the lesion to progressively protrude into the vessel and to reduce the lumen. In the case of acute events, instead, the plaque undergoes structural alterations resulting in the thinning of the fibrous cap (unstable plaque). This situation makes the unstable plaque prone to rupture and to loose the lining endothelium, thus allowing the highly pro-coagulant content of the lesion to get in contact with blood, triggering thrombus formation and acute vessel obstruction. The presence of an unstable plaque can be associated with characteristic clinical syndromes, such transient ischemic attacks, which precede the critical events [3, 11].

INFLAMMATION AND THROMBOSIS AS A SOURCE OF BIOMARKERS

The mature atherosclerotic plaque consists in an intra- and extracellular accumulation of oxidized lipids within the arterial wall, surrounded by a variable amount of fibrous tissue; however, it is now clear that the biochemical, molecular, and cellular events triggering its onset and determining its progression, evolution, and eventually its instabilization and rupture are to be viewed in the context of the inflammatory response [12].

Inflammatory signals are responsible for the increased permeability of the endothelial cells that leads to enhanced insudation of LDL into the arterial wall, for the migration, proliferation, and differentiation of inflammatory cells and fibroblasts driving the oxidation of LDL, the formation of the necrotic gruel and its fibrous cap, for the release of proteases and their tissue inhibitors driving plaque remodelling, and in general for the key events determining the different evolution of the plaque towards stabilization or instabilization and rupture [12]. On the other side, through the regulation of the production of plasma proteins involved in the blood clotting and fibrinolysis, the systemic inflammatory response influences also the liability of blood to sustain the thrombo-embolic events occurring in correspondence of the unstable plaque, that finally determine the clinical surfacing of the disease [13-15].

Taking this into account, it is not unexpected that several biomarkers reflecting the inflammatory process have been associated to atherosclerotic disease and its progression [13-17]; unfortunately, inflammation is a common response to all pathogens, and the signals and mechanisms driving inflammatory response are not tissue-specific, nor are specific for any

inflammation-triggering agent, including oxidized LDL, i.e., the inflammatory trigger of atherosclerosis. Besides, inflammation is always a systemic response due to the endocrine action of cytokines; for these reasons the search for atherosclerotic inflammation markers has led to the identification of several indicators associated to increased risk of acute cardiac and cerebrovascular events, yet none specific.

Among those proposed, there are primary inflammatory markers such as cytokines, which trigger (TNF- α , IL-6) or modulate (IL-10) the whole inflammatory process, as well as secondary markers, e.g., adhesion molecules (CAM) whose expression in endothelial cells mediates the migration of inflammatory cells into the arterial wall, or the acute phase proteins such as serum amyloid A component (SAA), or C-reactive protein (CRP), or other proteins produced by activated macrophages such as the Pregnancy Associated Plasma Protein A (Figure 1).

At the moment, C-reactive protein is the most studied inflammation marker in the setting of atherosclerosis. CRP is a pentraxin produced by hepatocytes during inflammation, but is also produced by lipid-laden macrophages in atherosclerotic plaques, thus suggesting that slight but persistent elevation of serum CRP reflects the individual atheromatous burden. Besides, since CRP is an activator of the complement cascade, the finding that the complement membrane attack complex is activated inside the plaque suggests that the intra-plaque production of CRP sustains an auto-toxic mechanism which acts within the plaques promoting cell death and acute thrombotic events, thus linking plasma levels of CRP with key events on plaque progression [18]. Indeed, in line with these findings, CRP appears to be an independent predictor of acute cardiovascular events both in asymptomatic subjects [19, 20] and in patients with unstable angina [21]; moreover, elevated CRP levels are a predictor of a worse prognosis in ischemic stroke patients [22, 23].

As regards haemostatic markers, it should be taken in mind, as stated above, that many proteins involved in coagulation and fibrinolysis are acute phase reactants, i.e., proteins, such as fibrinogen, whose plasma levels increase during inflammation due to the effect of inflammatory cytokines on gene expression in hepatocytes; fibrinogen [24] and D-dimer [25] have been proposed as biomarkers of unstable plaque (Figure 1). While fibrinogen is produced in the liver under the stimulation of cytokines, the D-dimer derives from the hydrolysis of fibrin by plasmin, thus is a marker of the fibrinolytic processes and of thromboembolic events. D-dimer levels correlate also with blood levels of CRP and serum amyloid A (SAA) [25], thus it can also be considered as a marker of inflammation [26]. D-dimer levels in blood have also been associated with increased risk of cardiovascular events in asymptomatic subjects [23, 27], of ischemic and cardio-embolic stroke [28, 29]. A recent retrospective study evaluated the diagnostic value of the serum biochemical markers CRP, D-dimer and fibrinogen in differentiating etiological subtypes of ischemic stroke basing on the TOAST classification system. CRP and D-dimer levels were significantly different among the subtypes and were the highest in cardioembolism (CE), followed by large- and small-artery occlusion; no significant difference between the subtypes was found for fibrinogen. Thus the combined evaluation of CRP and D-dimer serum levels could be useful for identifying the etiological subtypes of acute ischemic stroke, especially for predicting CE (CRP > 6.96 mg/L and D-dimer > 791.30 ng/mL: sensitivity 65%, specificity of 91%) [30].

Inflammation has not only a role in the pathogenesis of atherosclerosis, i.e., a main cause of stroke, but also in the tissue response to ischemic or haemorrhagic damage; for this reason,

inflammatory biomarkers (notably the same biomarkers mentioned above) have a promising value in predicting the short term and long-term prognosis of stroke.

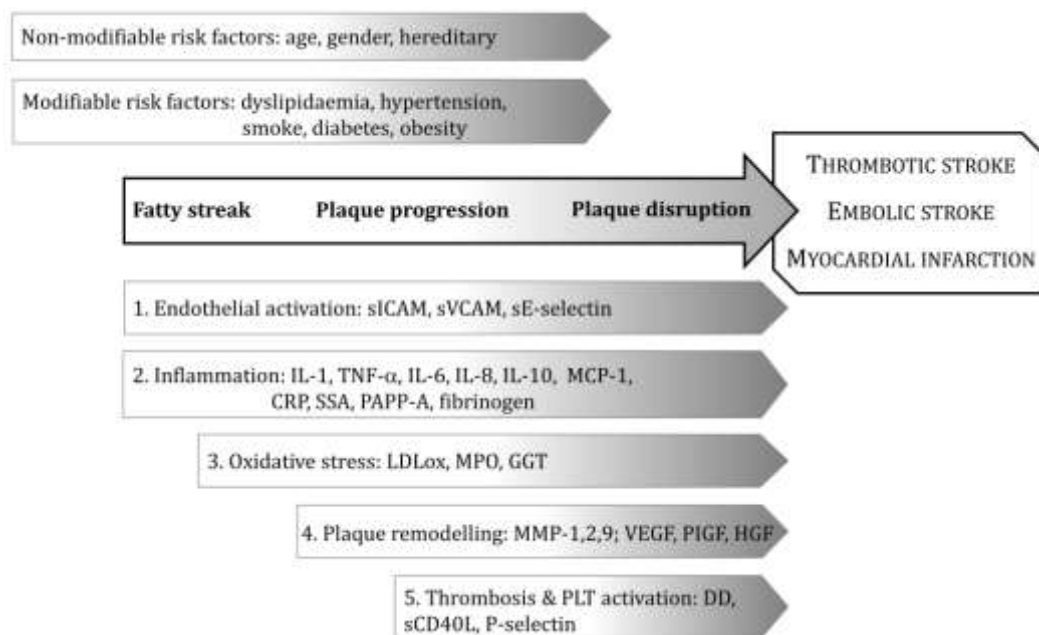


Figure 1. The diagram shows the main modifiable and non-modifiable risk factors as well as the pathophysiological sequence of plasma biomarkers corresponding to atherosclerotic plaque formation, progression and destabilization. 1. Endothelial activation: soluble forms of adhesion molecules may indicate the extent of endothelial cell activation (sICAM soluble intercellular adhesion molecule; sVCAM soluble vascular cell adhesion molecule, soluble E-selectin). 2. Inflammation: interleukin (IL-1; TNF- α ; IL-6; IL-8; IL-10), chemokine (MCP-1, monocyte chemotactic protein 1), and acute-phase reactants (fibrinogen; CRP, C-reactive protein; SSA, serum amyloid A; PAPP-A, pregnancy-associated plasma peptide A) are markers for basal inflammation that leads to development of atherosclerotic plaque. 3. Oxidative stress promotes LDL oxidation (LDLox), the development of foam cells and the progression of atheroma (MPO, myeloperoxidase; GGT, gamma-glutamyltransferase). 4. Plaque remodelling: inflammation promotes angiogenesis (VEGF, vascular endothelial growth factor; PlGF, placental growth factor; HGF, hepatocyte growth factor), activation of MMPs (matrix metalloproteinases, including MMP-1, -2, and -9), and release of PAPP-A (pregnancy-associated plasma peptide A) from macrophages. 5. Rupture and/or erosion of the unstable plaque leads to thrombosis and platelet activation (DD, D-dimer; sCD40L, soluble CD40 ligand; P-selectin).

In particular, if considering the short term prognosis, it is now clear that the exacerbation of the inflammatory response, which is substantiated by higher serum IL-6, CRP and SAA, is followed by increased in-hospital mortality for ischaemic stroke. If considering that inflammation is in itself a process aimed to destroy pathogens, rather than to protect the tissue, it is reasonable to suppose that the unbalanced action of anti- and pro-inflammatory cytokines enhances the potentially damaging side of inflammation, i.e., production of free radicals, microvascular damage, activation of proteases, chemotaxis of inflammatory cells; thus cytokines, the key players in the inflammatory mechanism, contribute to the progression of ischemic damage [31]. Microglia, astrocytes, endothelial cells, and neurons are the main cell types in the brain able to secrete pro-inflammatory and anti-inflammatory cytokines in

response to damaged tissue after ischemia [32], and notably not only the increased production of pro-inflammatory cytokines (TNF- α , interleukin (IL)-1 β , IL-6) is associated to worse prognosis, but the impaired production of anti-inflammatory cytokines (such as IL-10) is correlated with a larger infarct size in animal models and a possible worse clinical outcome in humans [33].

It is not yet clear whether the association between cytokine levels and the prognosis of stroke reflects a direct role of them in its clinical evolution, or it is only an epiphenomenon of the extent and severity of the nervous tissue damage; it should be taken in mind that the biological action of cytokines is not a direct consequence of their concentration in blood: in fact the biological action of the main cytokines, such as IL-1 and TNF- α is finely tuned by the combined effects of several ligands and receptors, that make difficult to establish a direct relationship between the plasma concentration of each cytokine with its real biological action at tissue level. A better understanding of this key aspect of brain damage is needed to allow a clinical application of this promising research line, which to now has produced partially conflicting findings when using cytokine serum levels for predicting outcome and infarct size [31].

OXIDATIVE STRESS AS A POSSIBLE SOURCE OF BIOMARKERS

Oxidative stress plays a key role in the aetiopathogenesis and in the modulation of the inflammatory process; in fact, many reactive oxygen species (ROS) are produced by inflammatory cells as mediators of the inflammation processes. Oxidative stress is also involved in the genesis of plaque, as ROS contribute to the oxidation of low-density lipoproteins (LDL), which, in turn, trigger the inflammation within the vessel wall (Figure 1) [2, 34].

The oxidation of LDL was highlighted by earlier studies as the single crucial event in pathogenesis of atherosclerosis, with special reference to the initiation of the lesions [2]. Subsequent research has shown however that the implication of oxidative processes in atherosclerosis may be considerably wider, including effects on several other important pathological changes occurring in the diseased vessel wall. Redox reactions are in fact involved in stimulation of smooth muscle cell proliferation, contributing to lesion thickening and expansion. Besides, the activities of matrix metalloproteinases and of their inhibitors, major determinants of plaque stability, are modulated through redox changes in critical portions of their protein structure. Some aspects of endothelial dysfunction (e.g., impairment of nitric oxide production, expression patterns and levels of adhesion molecules) are also affected by oxidative events, and several other redox-sensitive steps are present in signal transduction, apoptotic cell death and platelet functions. Altogether, current experimental studies consistently indicate that redox processes may contribute to turn a mildly stenotic, silent plaque into a vulnerable, symptomatic and potentially fatal lesion. A series of enzyme activities, found in cellular elements present in the lesions (endothelial cells, phagocytes, smooth muscle cells, fibroblasts) have been proposed as sources of the oxidants involved; these include NADPH-oxidases, NO-synthase, xanthine oxidase, myeloperoxidase, lipoxygenases and enzymes of mitochondrial respiration.

Since 1995, growing attention is dedicated to γ -glutamyltransferase (GGT) [35] whose levels in serum have been recently come on stage as a novel marker for cardiovascular prognosis related to atherosclerosis. The potential role of GGT in the pathogenesis of atherosclerosis is independently suggested by the biochemical demonstration of the production of free radical and reactive oxygen species as a by-product of GGT activity, but also by epidemiological and histochemical studies.

SERUM GAMMA-GLUTAMYLTRANSFERASE: A PROGNOSTIC MARKER IN CARDIO - VASCULAR DISEASES

The determination of serum or plasma levels of GGT is one of the basic biochemical tools for evaluation of liver function. The activity of GGT in blood is a sensitive biomarker of liver disease. As such, serum GGT activity is the parameter of choice for monitoring alcohol abuse and its association with morbidity was initially reported in this perspective [36].

Conigrave and collaborators [37] were the first to appreciate that GGT has a predictive value irrespective of hepatic disease or alcohol consumption. With respect to atherosclerotic diseases, it was observed that serum GGT levels were positively associated with increased risk of myocardial infarction [38] and more recently, the association between serum GGT and the risk of stroke was also observed [39-42].

These associations are partly explained by the known correlations between serum GGT levels and known cardiovascular risk factors, such as changes in blood lipid [39], body mass index [43], hypertension [44], glucose intolerance [45], insulin resistance [46] and type 2 diabetes [47]. GGT levels also correlate positively with novel risk factors such as C-reactive protein, fibrinogen and F2-isoprostanes [48]. It is worth emphasizing that most of the studies mentioned above refer to serum GGT values within the laboratory reference range, which would not otherwise raise specific health concern.

Of particular interest, the study by Wannamethee and collaborators [38] focused the relationship between GGT and ischemic heart disease (IHD). In a large prospective study (7613 middle-aged men, with a 11-years follow-up), GGT levels in the normal range were strongly associated with all-cause mortality, and the association was largely due to a significant increase in deaths from ischemic heart disease in the top quintile of GGT distribution. Serum GGT was positively correlated with pre-existing ischemic heart disease, diabetes mellitus, antihypertensive medication, systolic and diastolic blood pressure, total and HDL cholesterol, heart rate and blood glucose, while a negative association was found with physical activity and lung function. After adjustment for these variables, elevated GGT (highest quintile, ≥ 24 U/l, vs. the rest) was still associated with a significant increase in mortality from all causes and from IHD. The increased risk of IHD mortality was more marked for patients with evidence of ischemic heart disease at screening, particularly those with previous myocardial infarction; these findings clearly pointed to a connection of GGT with underlying atherosclerotic coronary artery disease.

The supposed connection of GGT with atherosclerotic disease has received a detailed assessment in a prospective study of Emdin and collaborators [49]. The study included a six-year follow-up of 469 patients with ischemic syndrome and angiographically documented coronary artery disease (CAD). After correction for other cardiovascular disease risk factors

(age, smoking, serum cholesterol, left ventricular ejection fraction, body mass index, diabetes mellitus), or confounding factors (serum alanine aminotransferase, self-reported alcohol consumption), the prognostic value of serum GGT activity for cardiac death and non-fatal infarction was confirmed. In particular, the significance of serum GGT was more evident in a subset of patients prone to plaque complications, i.e., characterized by the association of diffuse atherosclerosis (“multivessel disease”) and a history of previous myocardial infarction (approx. 36% of the whole population). The risk was increasing using two different GGT cut-off values (25 or 40 U/L, both considered within the normal range), and the event excess was concentrated within the first three years. The prognostic significance of serum GGT was thus correlated with the diffusion of coronary artery disease. Interestingly, the significance of serum GGT appeared to depend on the instability of plaque as well, as indicated by the fact that the prognostic value of GGT disappeared after revascularization by angioplasty, i.e., a procedure causing plaque stabilization [50]. Thus, the unfavourable prognosis signalled by elevated serum GGT seems to apply specifically to patients with vulnerable plaques, suggesting that connections of some kind must exist between GGT and the processes involved in plaque instability. Moreover, GGT, CRP and fasting glucose show an additive prognostic value; in fact, high levels of these biomarkers identify a subset of patients with the highest risk of vascular events [51].

The study by Ruttman and collaborators [52], carried out in as many as 163,944 Austrian adult subjects, has now rather conclusively shown that serum GGT levels are an independent prognostic factors for fatal events of chronic forms of CAD, congestive heart failure and ischemic or haemorrhagic stroke. The finding was found to be true for both-sexes, with a clear dose-response relationship and a stronger significance as far as younger subjects were concerned. Ruttman and colleagues established the prognostic value of GGT for cardiovascular events at serum levels that lie within normal values. The receiver operating characteristics analysis suggested GGT cut-off values of 15.5 U/L for men and 10.5 U/L for women, corresponding to 27.6 U/L and 18.7 U/L, respectively, for current measurements made at 37°C.

In the last year other prospective studies, included the Framingham heart study [53-55], and meta-analysis confirmed the association between GGT and cardiovascular [56] or cerebrovascular disease [42] related to atherosclerosis.

GAMMA-GLUTAMYLTRANSFERASE INSIDE PLAQUES: POSSIBLE CONNECTIONS WITH SERUM GGT

Epidemiological studies suggest a relationship between circulating GGT and the evolution of atherosclerotic plaque, but the pathogenetic mechanism has not been elucidated yet. A clue was independently provided by studies showing the presence of GGT inside atherosclerotic lesions. Histochemical studies, in fact, revealed intense GGT activity in the intimal layers of human atherosclerotic lesions, apparently localised in CD68+ macrophage-derived foam cells [57, 58]. GGT-positive cells were found to co-localise also with immunoreactive oxidized LDL [59], and catalytically active GGT could also be detected in micro-thrombi adhering to the surface of atheromas [60].

GGT catalyses the first step of extracellular glutathione (GSH) degradation, i.e., the hydrolysis of the gamma-glutamyl bond existing between glutamic acid and cysteine, in this way GGT allows to use extracellular GSH as a source of cysteine and amino acids for the synthesis of intracellular GSH [61]. In pathological conditions, these reactions may have important pro-oxidant implications [62]. In fact, cysteinyl-glycine, the dipeptide that originates from hydrolysis of GSH, is extremely efficient in transferring electrons to metal cations, thus triggering a redox cycling capable to catalyse the production of free radicals and ROS [62, 63]. These reactions have been shown to take place inside plaques, thus GGT activity might contribute directly to the oxidative events influencing plaque evolution and rupture. This hypothesis was confirmed by the finding that morphologically unstable plaques contain more GGT activity [64].

In order to understand the precise role of the GGT in plaque instability, it is necessary to understand how serum GGT levels reflected GGT activity and function within the plaque. The origin of GGT activity detected in atherosclerotic plaques is still unclear. A possible source is represented by macrophages; in fact, it has been shown that GGT is over-expressed and released by the pro-inflammatory M1 macrophages [65] present in the active plaques. Anyway, the correlation between GGT activity found within plaques and in serum suggests also an exchange of GGT activity between the two compartments [64]. Another line of evidence points to the possibility that the source of serum GGT may be identified in circulating platelets, as the two parameters are usually correlated. Thus, it has been proposed that the increased GGT levels observed in atherosclerotic patients may originate from increased platelet consumption at the site of plaques [66]. A recent report, pointing to a prognostic value of elevated serum GGT in stent restenosis, in fact appears to support the potential role of platelets as a source of circulating GGT [67].

GGT in blood is carried in macromolecular complexes (fractions) whose chemical and physical properties have partially been defined [68]. Four GGT fractions have been detected in plasma of all healthy subjects by size exclusion chromatography [69]: b-GGT (2000 kDa), m-GGT (940 kDa), s-GGT (140 kDa) and f-GGT (70 kDa). In healthy subjects, only b-GGT levels are clearly associated with the main cardiovascular risk factors (i.e., body mass index, systolic blood pressure, serum glucose, serum cholesterol, serum triglycerides) as well as with inflammatory biomarkers (leukocyte count, C reactive protein) at difference with other fractions, and in particular f-GGT, the main fraction found in healthy individuals [70]. Furthermore, only b-GGT was found in human atherosclerotic plaques, co-localized with products deriving from the pro-oxidant reactions catalysed by GGT activity, thus suggesting that only this fraction, rather than total GGT itself, may be responsible of the association between GGT [64] levels and atherosclerotic disease. Besides, in a group of patients subjected to carotid endoarterectomy, a significant correlation was found between serum and intra-plaque b-GGT activity [64].

The physical features of the molecular complex corresponding to b-GGT are those of membrane microvesicles, likely microparticles and/or exosomes; this finding opens new perspectives on the role of serum GGT on atherosclerotic-related events [68].

Microparticles are nowadays considered main characters in cell communication as well as in the modulation of inflammation and haemostatic balance; microparticles expose tissue factor and phosphatidylserine that confer the pro-coagulant properties to microparticles [71]. Several studies have documented increased platelet, leukocyte or endothelial-derived microparticles in patients with cardiovascular disease [72]. Further prospective studies will

clarify the prognostic value of pro-coagulant microparticles in the prediction of thrombotic events. A challenging question is whether microparticles can be used as a marker of the haemostatic balance of blood that would be of help to identify “vulnerable” patients. In fact, the consequences of plaque rupture depend both on the solid state of the atheroma and the “fluid” phase of the blood that can predispose toward coronary thrombosis.

Future studies will clarify if serum b-GGT activity represent a marker of the atherosclerotic burden, suggesting the presence of a vulnerable plaque, or of the blood coagulant activity, thus indicating a vulnerable patient.

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Chapter 37

MITOCHONDRIA, MITOCHONDRIAL DNA AND STROKE

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ABSTRACT

Stroke may represent the clinical expression of several inherited disorders, including the mitochondrial disease Mitochondrial Encephalomyopathy, Lactic Acidosis and stroke-like episodes (MELAS). Moreover, converging evidences link together inherited or acquired mitochondrial dysfunction and stroke, and provide intriguing therapeutic implications in terms of neuroprotection.

Here we revise the available knowledge linking together inherited and acquired mitochondrial dysfunction and stroke.

Keywords: stroke, genetics, mitochondrial disease, mtDNA, MELAS

INTRODUCTION

Multiple evidences suggest that the etiopathogenetic mechanisms of stroke have not been fully understood; the conventional vascular risk factors, however, do not entirely account for the incidence of stroke in unexposed populations, and more than one-fourth of cases, particularly strokes in young patients, are classified as cryptogenic [1]. Consistently, twin and family history studies suggest a role for genetic factors in stroke risk that could be

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particularly relevant especially in young patients and non-cardioembolic ischemic stroke [2, 3]. A full review of the stroke associated genetic disorders goes beyond the scope of this chapter, and has already been treated also by our group [4]. Instead, we want to focus our attention on the emerging evidence linking together inherited and acquired mitochondrial dysfunction and stroke.

WHY MITOCHONDRION?

According with the WHO definition, stroke is a cerebrovascular accident caused by “the interruption of the blood supply to the brain (...). This cuts off the supply of oxygen and nutrients, causing damage to the brain tissue.” In order to fully understand the axiom, we have to enter into the physiological and ultrastructural mechanisms of stroke pathogenesis. Since the sudden interruption of oxygen and oxidizable substrates first impairs the ability to meet the high energy demand of neuronal cells, it is therefore appropriate to analyze the main source of cellular energy, the mitochondria. Although the Central Nervous System (CNS) represents only 1-2% of the body weight, it receives, in basal conditions, approximately 15% of the cardiac output; therefore, the cerebral blood flow is typically 750-1000 millilitres per minute. The complex and delicate balance of the biochemical and electrophysiological networks that underlie neuro-neuronal and neuro-glial connections are based on the chemical energy burst from the phospho-derivate products of anaerobic and, mostly, aerobic glucose metabolism. A peculiar feature of neuronal cells, the excitability, requires correctly functioning membrane wall Na^+/K^+ ATPase pumps, intracellular Ca^{2+} -concentration regulation, intra and extra-cellular signaling molecules, vesicles transporting, and cellular differentiation [5, 6].

This background clearly suggests a pivotal role of the mitochondria on CNS functions, as demonstrated in different disease models [7, 8, 9]. Indeed, the mitochondrion is currently known to play a major etiopathogenetic role in many human diseases and processes: neuromuscular and neurodegenerative disorders (i.e., Parkinson’s and Alzheimer’s diseases), diabetes mellitus, aging, programmed cell death and carcinogenesis, to name a few [5, 10, 11, 12, 13].

OVERVIEW OF MITOCHONDRIAL BIOLOGY AND GENETICS

More than a billion years ago, aerobic bacteria colonized primordial eukaryotic cells that were not able to use oxygen. This symbiotic relationship could soon become permanent as bacteria evolved into mitochondria, providing aerobic metabolism to host cells. Mitochondria are ubiquitous in eukaryotes and are essential for their survival, representing the cellular powerhouse that generates ATP, a chemical energy source, through a chain of reactions known as oxidative phosphorylation (OXPHOS) [5, 10].

Mitochondria are surrounded by two distinct membranes, an outer (OMM) and an inner membrane (IMM) that invaginates to form the “cristae”. The mitochondria form complex networks by virtue of their ability to move, fuse, and split. There are tissue-specific differences in mitochondrial activity and biogenesis, mainly related to the tissue dependence upon oxidative phosphorylation for energy provision: neurons, cardiac and skeletal muscles

have high mitochondria density that explains their vulnerability to energy-dependent defects and injuries resulting from mitochondrial dysfunction.

Human mitochondrial DNA (mtDNA) is a small circular, double-stranded molecule of 16,569-kb, which contains 37 genes: 2 rRNA genes, 22 tRNA genes, and 13 structural genes encoding subunits of the mitochondrial respiratory chain, the ‘gas station’ of oxidative metabolism, where ATP is synthesized [10, 12, 13, 14]. Reducing equivalents from the Krebs cycle and the β -oxidation spirals are passed through several protein complexes (the electron transport chain –ETC–) embedded in the IMM, and consisting of four multimeric complexes (I to IV) plus two small electron carriers, coenzyme Q and cytochrome c. The energy generated by the reactions of the ETC is used to pump protons from the mitochondrial matrix into the space between the mitochondrial membranes, in order to maintain an electrochemical proton gradient, that is used by complex V (the ATP synthase) to generate ATP as protons flow back into the matrix through its membrane-embedded F₀ portion, the “rotor” of the turbine [12, 14].

Mitochondria descend exclusively from the oocyte; therefore, mtDNA is maternally-inherited. Cells have variable amount of mitochondria, each of which contains multiple (polyplasmcy) and identical (homoplasmy) copies of mtDNA. Mutations in all the mtDNAs are defined homoplasmic. Differently, heteroplasmic mutations, which are harboured by only a part of mtDNA molecules, are often responsible for disease phenotypes [12, 14]. Molecules of mutated and wild-type mtDNA coexist in the same cell and the mutant genomes are randomly distributed to the daughter cells; therefore, their amount is different in the various mitotic cycles (different mitotic segregation). Symptoms will appear when the mutated molecules will be enough to cause energetic crisis (threshold effect), and it’s worth to consider that the percentage of mutated genomes able to manifest a phenotype varies in different tissues. Age of onset and clinical picture will depend on the amount of mutated genomes and on their distribution in the different tissues. Tissues particularly dependent on oxidative metabolism, and/or characterized by a low mitotic rate (i.e., skeletal and cardiac muscles, central nervous system, liver and kidney) appear more vulnerable to alterations of the mitochondrial function; therefore, a relatively small amount of mutated genome may be sufficient to cause phenotype.

Beyond energy metabolism, mitochondria are also involved in iron-sulfur cluster assembly, intracellular Ca²⁺ concentration, ROS signaling, apoptosis, immunity and neuronal plasticity [15, 16].

MITOCHONDRIAL DISEASE

Clinically, the most important consequence of mitochondrial genetic complexity is the extreme heterogeneity of the phenotypes associated with mitochondrial defects.

The same genetic defect may result in different clinical pictures, also within the same family and, by contrast, similar phenotypes may be the expression of different mutations (either nuclear or mitochondrial). Moreover, phenotypes are extremely polymorphous, and may range from pure myopathies to multisystem disorders, making difficult to draw definite genotype/phenotype correlations.

At the molecular level, mitochondrial encephalomyopathies are classified as those due to mutations in mtDNA, which cause maternally inherited or sporadic disorders, and those due to mutations in nuclear DNA (nDNA), Mendelian inherited. A more practical framework for categorizing these diseases distinguishes three groups: mtDNA defects; nDNA defects that directly or indirectly affect the respiratory chain; defects of mtDNA maintenance (impaired intergenomic communication) [17]. Aim of this work is not to provide a comprehensive revision of the different clinical manifestations of mitochondrial diseases, already revised elsewhere [17]. We here focus our attention on the mitochondrial stroke-like disease, the MELAS syndrome.

MELAS, A CEREBROVASCULAR MITOCHONDRIOPATHY

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (OMIM 540000), is a multisystem disorder with a clinical onset usually in the first two decades of life. It consists of a progressive encephalomyopathy characterized by repeated stroke-like events (SLE) mainly involving posterior cerebral areas. Typically, the SLE are characterized by the acute onset of neurological dysfunction without a corresponding classical vascular distribution, being the irregular distribution more consistent with a metabolic endothelial dysfunction of small vessels [17]. The single episode may lead to reversible aphasia, hemianopia, cortical blindness, but accumulating irreversible neurological deficit and radiological burden become prominent; in fact, the cumulative residual effect of SLE results in impaired motor abilities, vision and cognition. CNS areas typically involved are, asymmetrically, temporal, parietal or occipital lobes, whereas deep white matter is relatively spared. Diffusion-weighted (DWI) MR-sequences are the gold standard imaging techniques for the characterization of these infarct-like metabolic lesions. Although SLE typically involve small vessels, some reports suggest a role in large vessel occlusion by thrombus formation in an abnormal endothelium or arterial dissection (very rare). The pathogenetic mechanisms leading to SLE are yet not fully understood, and two main hypotheses are being considered: (i) the neuronopathy hypothesis: impaired OXPHOS results in high potassium and glutamate in the synaptic cleft, and the consequent neuronal hyperexcitability may clinically manifest with SLE, seizures or migraine; (ii) the angiopathy hypothesis: histological analysis in MELAS revealed increased enlarged mitochondria with complicate cristae in pericytes of capillaries, endothelial cells and smooth muscle cells of pial vessels and small intracerebral arteries [19, 20]. This pathological data may represent a compensatory mechanism to overcome the OXPHOS defect, and SLE may thus occur when this compensatory reaction does not meet the increasing demand of a brain region. Impaired vasodilatory mechanisms (a feature of small vessels dysfunction) have been reported in MELAS patients. Finally, changes in the nitric oxide (NO) pathway have been linked to SLE presentation and justify the use of L-arginine (a NO precursor) therapy during the acute metabolic crises in MELAS [21, 22, 23].

Recurrent migraine-like headaches and vomiting, exercise intolerance, seizures, ataxia, cardiomyopathy, sensorineural hearing loss, short stature and lactic acidosis are other frequent clinical features [1, 24]. The Italian Network of Mitochondrial Diseases has recently demonstrated that SLE in MELAS are tightly associated with lactate acidosis, generalized seizures, cognitive impairment, and hearing loss [18]; interestingly, a potential gender effect

has been postulated in this study [18]. In the Italian cohort, we observed that MELAS group related to the 3243A>G mutation in mtDNA was enriched in males compared with the non-MELAS group ($p = 0.019$); this observation was not explained by other possible confounding factors such as age of onset, age at the last control, disease duration, levels of heteroplasmy in tissues (muscle, blood and urine), that were not significantly different between the two groups. Even if this finding need to be confirmed through specifically designed longitudinal studies, gender effect is not a novel concept in mitochondrial diseases. An higher prevalence of Leber hereditary optic neuropathy in males is already known [25], and may be related to multilayer cytoprotective effects induced by estrogens [25].

A number of point mutations have been described in association with MELAS phenotype. Although some of them are located in polypeptide-encoding genes, especially ND (NADH dehydrogenase, Complex I), they more frequently arise in tRNA genes. The most commonly reported mutations associated with MELAS are the m.3243A>G and m.3271T>C in tRNA Leu (UUR) [17]. Multiple additional mutations may also cause MELAS [26].

The diagnosis of MELAS should be kept in mind when the following features are present: (i) stroke-like episodes which start typically before age 40, (ii) seizures, cognitive impairment or both and (iii) mitochondrial dysfunction as showed by the presence of lactic acidosis, ragged-red fibers or both. Conventional MRI and proton spectroscopy are important and may detect a lactate peak in both brain and CSF (cerebro spinal fluid), supporting a clinical suspect of mitochondrial disorder [27]. Molecular diagnosis is then necessary to confirm the clinical suspect of MELAS. Heteroplasmy levels of the m.3243A>G mutation are usually higher in muscle and urine than blood [18]. Therefore, urine sediment is a valid and easily accessible tissue for the detection of the m.3243A>G mitochondrial mutation.

THE VASCULAR CASCADE LEADING TO ISCHEMIA/REPERFUSION INJURY DURING ACUTE STROKE IS LINKED TO THE MITOCHONDRION: THE ROS STRATEGY AND THE OXYGEN PARADOX

So far, we focused our attention on inheritable mitochondrial diseases; however, and not surprisingly, growing evidences suggest that specific mitochondrial haplogroups, mitochondrial distribution, SNPs (Single Nucleotide Polymorphisms) variability, and de-novo aging-related mtDNA mutations/amplifications may influence risk, prognosis, and also the therapeutic response in acute or chronic CNS diseases, including stroke [7]. Cellular stress response consists in an “evolutionarily conserved mechanism” that activates molecular homeostatic-inflammatory pathways after exogenous and/or endogenous injuries [5, 28]. Cellular energetics, mitochondrial energy production and oxidative stress are crucial for cell stress adaptation and cell response; therefore, variable mitochondrial function, secondary to different genetic background, would differentially modulate the organism’s response to different injuries. Mitochondria are, in fact, dynamic organelles, and both function and morphology may vary within cellular type, developmental stage, and environment [29, 30]. Moreover, mtDNA appears more susceptible than nDNA to oxidative stress and environmental toxins [31] and it replicates continuously, independently from the cell cycle

[32]. During lifetime mitochondria may thus accumulate aging/environment related mutation(s) that, in turn, may lead to energy failure, ROS production and cell damage [33].

Mitochondrial function and ROS-homeostasis are paramount for vascular cell remodeling (proliferation and apoptosis), inflammatory response and atherosclerosis [5, 34, 35, 36, 37] and may favour atherosclerotic plaque rupture [38].

Given its high metabolic rate, brain appears particularly vulnerable to the deleterious effects of ischemia. The cessation of blood supply leads to ischemia and eventually cell injury by decreased availability of oxygen and other nutrients (first-direct ischemia hit). Ischemic injury results from severe impairment of cell metabolism through decreased ATP availability, subsequent loss of membrane potential, and calcium overload that leads to cell death through either necrosis, apoptosis or autophagy. During ischemia, loss of mitochondrial membrane potential and functional impairment of mitochondrial proteins have been observed, leading to cessation of oxidative phosphorylation and accumulation of ROS (reactive oxygen species), suggesting a pivotal role of mitochondria in the pathophysiology of ischemic injury [39, 40].

Restoration of blood flow after a sufficiently prolonged ischemia may result into further injury, as a result of early and delayed reperfusion injury; therefore, rather than an expected decrease of the injured zone, a larger zone may be observed (second-indirect ischemia hit).

In early reperfusion injury, oxygen may exacerbate brain damage through multiple mechanisms, such as activation and modulation of similar-apoptotic and necrotic pathways, overproduction of inflammatory mediators, stimulation of leucocyte diapedesis, and alteration of enzyme conformations. In this regard, mitochondrial proteins appear particularly vulnerable, and further mitochondrial damage may result in further ROS production and overwhelming stimulation of apoptosis and necrosis, in a vicious manner [40]. Delayed-reperfusion injury is instead related to the promotion of tissutal and vascular remodeling processes that finally result in tissue fibrosis and scar formation.

All these issues may be related to repeated bursts of ROS production that may be observed during cessation and subsequent recovery of oxygen delivery (ischemia/reperfusion -I/R- and post-reperfusion phases of I/R injury). The biochemical, pathological, and clinical consequences of this “oxygen paradox” are related to the type and amount of ROS, as well as the location in the cell, both for early and delayed reperfusion injury (ROS-related angiogenesis and cell division and differentiation regulation) [40]. A limited and circumscribed ROS production leads to less severe ischemic injury, whereas massive ROS production that involves all mitochondria may result in massive ischemic injury [40].

It is well known that mitochondrial respiratory chain complex I-III impairment is linked with I/R damage [39]. Moreover, deglutathione complex II and phosphorylated complex IV have been proposed as relevant sources of ROS. ROS induced ROS-formation in post-ischemic phase by the Krebs enzymes and NADPH oxidase (NOX) activation is suggested by in vivo models reporting decreased glial activation and neuronal cell degeneration following NOX modulation. Nox4 represents the most expressed isoform of NOX in brain tissue and its modulation by hypoxia related responses may not only influence the redox set point at cellular level, but also play a role in cell proliferation and differentiation during the later reperfusion phases [39, 40].

A pivotal role in the balance of the opposite tissue responses to O₂ delivery interruption/recovery has been proposed for mitochondria permeability transition pores (mPTPs): their short-term opening has been involved in cardioprotection (transient ROS formation), whereas long-lasting opening may irreversibly impair cellular homeostasis [41].

Growing evidence suggests that sub-ischemic damage may precondition brain tissue through the activation of specific cell surviving programs, and modulation of key enzymes involved in cellular death pathways. Ischemic injuries not reaching the “necrotic threshold” may thus result in brain protecting responses. Again, mitochondria and, specifically, ROS are well known leading actors in the pathways involved in these processes, and may play a crucial role in the balance between cell injury and protection from ischemic damage [40, 42].

TARGETING MITOCHONDRIA FOR STROKE THERAPY. PRECLINICAL STUDIES

Although extensive studies have shown that several neuroprotectants reduce infarction and improve neurological functioning in animal models of stroke, few of those agents have been successfully translated into clinical practice. This situation necessitates the exploration of novel neuroprotectants, such as ischemia postconditioning. Ischemic postconditioning initially refers to a series of brief ischemia and reperfusion cycles applied after reperfusion, which is a concept defined in contrast with ischemic preconditioning. While postconditioning is performed after reperfusion, preconditioning is conducted before the onset of ischemia [43]. In stroke research, the methods used for postconditioning include a stuttering reperfusion, meaning a series of intermittent hypoxia, a single period of ischemia or hypoxia. Cellular pathways involved in postconditioning converge to a common target, the mitochondrion, and a particular role may be played by ATP-dependent potassium channels, ROS, and mPTPs [44]. From the bench to the bedside, as reperfusion could paradoxically increase injury and tissue loss, mitochondrial-targeted ischemic postconditioning may potentially present significant implications for stroke management, in terms of decreased infarct volume, and better neurological outcome.

An early administered mitochondria targeted therapy may therefore support and eventually enhance the effectiveness of standard stroke treatments. Supporting energy production may have a positive impact on reducing damage caused by a stroke and may favour a good functional recovery.

Data from preclinical studies emphasize the importance of mitochondria as candidates for stroke neuroprotection, since mitochondrial metabolism enhancers have been related to better preserved penumbral tissues [45]. Up-regulation of ATP synthesis by purinergic receptor stimulation has been proved to be a possible strategy: binding of 2-methylthioadenosine diphosphate trisodium salt (2meSADP) to purinergic receptor P2Y₁R activates the inositol triphosphate (IP₃) cascade resulting in release of calcium (Ca²⁺) from endoplasmic stores of astrocytes, translating into increased ATP production within the mitochondria for the increased cellular demands following injury and neuroprotection [46]. This molecules enhance neuronal survival, reverse the beading and swelling of dendrites associated with neuronal death, repolarize mitochondrial membrane potential [47] and reduce lesion volume in a cerebral ischemia/reperfusion model (middle cerebral artery occlusion –MCAO- with 60 min reperfusion in rats [45]).

Furthermore, methylene blue (an alternative electron carrier shuttling electrons between NADH and cytochrome c) revealed to be effective in attenuating I/R syndrome [48], and

protective in a transient cerebral ischemia model using the intraluminal filament MCAO in rats [49].

GENETIC MITOCHONDRIAL VARIATION AND STROKE SUSCEPTIBILITY

Over the past years a growing interest on the correlation between common mitochondrial genetic variations (SNPs) and haplogroups and stroke risk (or stroke phenotypes) have been reported.

Mitochondrial haplogroups are common genetic polymorphisms within the mitochondrial genome, accumulated over time, that describe classes of continent-specific genotypes, evolved from the same ancestor, which can be detected by restriction fragment length polymorphism (RFLP) analysis. It has been supposed that these genetic polymorphisms might lead to impaired energy generation and to increased amount of ROS, causing subtle differences in the encoded proteins and, thus, minimum changes in mitochondrial OXPHOS activity and free radical overproduction [50].

Previous evidences show that these genetic variability may influence the penetrance of mitochondrial genetic disorders, longevity, cancer, fertility, diabetes, hypertension, neurodegenerative diseases, but also cardio and cerebrovascular diseases, with a complex interaction between classical risk factors and genetic influences [51, 52, 53, 54, 55, 38, 13].

Studies on the role of mitochondrial haplogroups in stroke have produced conflicting results, although the trend seems to confirm an involvement of these variants as predictors of both clinical outcome and treatment response.

Two elegant studies carried out by Anderson and colleagues [56, 57] matched together autosomal and mitochondrial OXPHOS SNPs and stroke risk through a stroke genetic risk score. Interestingly, common mitochondrial genetic variants or multiple rare variants may interact to increase over-life stroke risk and white matter hyperintensity volume [56]. Moreover, an association between common genetic variants in OXPHOS genes and small vessel ischemic and hemorrhagic stroke has been demonstrated [57].

Biffi and colleagues [58] applied the same stroke genetic risk score demonstrating significant associations between common OXPHOS DNA sequence variants and clinical and radiographic markers of Alzheimer's Disease (AD), suggesting an overlapping genetic architecture between small vessel stroke and AD [58].

Specific haplogroups have also been found to be stroke-correlated. Majamaa and colleagues reported haplogroup U as a risk factor for occipital stroke in patients with migraine, in whom the mtDNA m.3243A>G mutation has previously been excluded [59]. In a Japanese study performed on 1081 patients in 2007, Nishigaki demonstrated an association between haplogroups A and atherothrombotic cerebral infarction in women, but not in men [60]. Some specific haplotypes seem also to play a protective role: in a Portuguese study on 534 ischemic stroke patients and 499 controls, haplogroup H1 was found to be significantly less frequent in stroke patients compared with controls [38]; similar results were observed for haplogroup D4 in a Chinese population [61]. Finally, Chinnery and co-authors demonstrated the protective role of sub-haplogroup K for both transient ischaemic attack (TIA) and ischaemic stroke, but not for coronary ischaemic vascular events [62].

However, a recent study on 9254 individuals from the Danish general population did not revealed an association of any mitochondrial haplogroups with risk of ischemic cardiovascular events [55].

For the geographical mtDNA characteristics, these conflicting results may be due to methodological issues, such as differences in study design, and in the descent of study populations.

A recently published study focuses on the possible impact of mtDNA haplogroups on short-term outcomes of neurological functions. 303 Chinese patients were investigated for changes between baseline and 14-day follow-up stroke severity, according to the National Institutes of Health Stroke Scale and the modified Rankin Scale scores, adjusting for age, hypertension, diabetes mellitus, TOAST subtype, antihypertensive treatment, glucose and cholesterol-lowering treatment. The Authors found that haplogroup N9 was an independent protective factor against neurological worsening [63].

CONCLUSION

Current treatment of stroke is focused to quickly restore blood flow to the damaged brain area, although this not only cannot be sufficient to restore normal neuronal functions, but may also result in worse functional outcomes. Converging evidence highlights the mitochondrial involvement in vascular diseases, focusing on several mito-footprints in the ischemic/reperfusion damage pathway. Mitochondrial involvement has been proposed in the pathophysiology of acute stroke, but the exact role for the mitochondrion and the mtDNA has not been comprehensively studied. Population-based analysis, as well as cell and animal models, have demonstrated that mitochondria may represent a potential target in stroke prevention and treatment, and also in post-stroke neurological recovery.

As the founder of the scientific method claimed more than 400 years ago the key must be sought in “this great book which stands continually open to our eyes (I mean the universe),” stroke researchers should analyze what already exists standing continually open to their eyes: the mitochondrion and the complexity of its machinery.

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Chapter 38

CERVICAL ARTERY DISSECTION: WHAT'S NEW IN 2015

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ABSTRACT

Cervical artery dissection (CeAD) is a major cause of ischemic stroke in young and middle-aged adults, but the incidence in the general population is low. While non-invasive methods for diagnosing CeAD have been substantially improved in the past two decades, the mechanisms of this potentially severe condition remains incompletely understood. Moreover, evidence based therapeutic strategies are lacking. Interestingly, in the past few years large international collaborative projects provided important novel information on environmental and genetic risk factors of CeAD, and led to new insight on the link between CeAD and other vascular diseases. Recently, the first phase 2 randomized trial of CeAD comparing antiplatelet agents to anticoagulants was published. This review aims primarily at summarizing novel findings regarding risk factors and treatment of CeAD.

Keywords: cervical artery dissection, ischemic stroke

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INTRODUCTION

Cervical artery dissection (CeAD), caused by a hematoma in the wall of the cervical portion of an internal carotid or vertebral artery, and is a major etiology of ischemic stroke in young and middle-aged adults [1-3]. The incidence of the disease is relatively low in the general population, estimated around 3/100,000 inhabitants per year [4, 5]. The mean age of occurrence of CeAD is 44 years. Carotid dissections are more common than vertebral dissections in populations of European origin [5], while a predominance of vertebral dissection has been described in Asian cohorts [6, 7]. A slight male predominance was reported in European (but not North-American) cohorts, and is more marked in Asian populations [1, 8]. Men more often suffer from carotid dissection and are on average 5 years older than women at the age of CeAD occurrence [1, 9, 10].

In published series about 67-77% of CeAD patients suffer from ischemic stroke or transient ischemic attack, which may be an overestimation, since large CeAD cohorts were recruited through departments of neurology specialized in stroke care [1]. Other ischemic manifestations include retinal ischemia in case of carotid dissection and seldom spinal cord ischemia in patients with vertebral artery dissection. Cervical pain and headache are the most common symptoms, and may precede the onset of cerebral ischemia by days or sometimes weeks. Horner syndrome can occur in patients with carotid dissection, due to compression of sympathetic fibers by the enlarged artery. Other complications include tinnitus, cranial nerve palsy and, seldom, subarachnoid hemorrhage (mostly when the dissection extends intracranially).

Radiologically, pathognomonic signs of CeAD include the presence of a mural hematoma (best seen as a crescent-shaped hyperintensity on T1-weighted axial magnetic resonance images), or more seldom a double lumen, or an intimal flap. On angiography - now mostly magnetic resonance or computed tomography angiography - CeAD most often presents as a long, irregular stenosis. CeAD can also present as an arterial occlusion or a dissecting aneurysm. In patients with arterial occlusion, if a mural hematoma cannot be seen, the diagnosis of CeAD can only be confirmed if during follow-up the occlusion recanalizes and leads to a typical stenosis or a dissecting aneurysm. In ~15% of the cases, CeAD affects multiple arteries.

Although the pathophysiology of CeAD is incompletely understood, data from large hospital-based cohorts of CeAD patients have provided new insight into the environmental [11], and more recently the genetic risk factors of CeAD [12]. Moreover, while evidence-based data for the management of CeAD is scarce, the first randomized feasibility trial comparing antiplatelet agents to anticoagulants in patients with CeAD was recently published [13]. We will summarize the main new findings emerging from these publications.

“ENVIRONMENTAL” RISK FACTORS AND VASCULAR COMORBIDITIES

Cervical trauma is an important risk factor for CeAD [14, 15]. Rarely, CeAD can occur after a major penetrating or blunt trauma. Often, in about 40% of the cases, CeAD patients report a cervical trauma in the days or weeks preceding the dissection [16], although a causal relationship is often difficult to prove. These are mostly minor traumas, i.e., not leading to a

medical visit or hospitalization. Cervical manipulation therapy and cervical trauma during sports are significantly more common in CeAD patients than in age- and gender-matched patients with an ischemic stroke due to other etiologies [16].

Interestingly, anatomic factors, such as reduced distance between the internal carotid artery and styloid and hyoid bones, were recently shown to be associated with a higher risk of carotid dissections [17, 18]. It could be speculated that these anatomical factors may increase the susceptibility to CeAD after minor cervical trauma, but this remains to be proven.

Recent infection has been reported as a potential risk factor or trigger of CeAD [19-21], and acute CeAD is associated with high white blood cell counts compared to patients admitted for non-CeAD ischemic stroke (IS), i.e., IS due to other etiologies, or to healthy controls [22], possibly reflecting a pre-existing inflammatory state. On cervical high-resolution magnetic resonance imaging, patients with spontaneous CeAD more often have periarterial edema, often in conjunction with elevated C-reactive protein or erythrocyte sedimentation rate, than patients with traumatic CeAD [23].

A seasonal pattern in the incidence of CeAD has been described, with a higher incidence in autumn and winter. This may be attributable to an increased prevalence of infection, higher blood pressure levels, and winter sport practice [24].

Migraine is a risk factor for CeAD, approximately doubling the risk – the absolute risk remaining extremely small, given the low incidence of CeAD [25-27]. While migraine is also a risk factor for ischemic stroke of other etiologies [28], it is even more common in CeAD patients compared to age- and sex-matched patients with non-CeAD IS [29]. Moreover, CeAD is more commonly associated with migraine without aura (in contrast with non-CeAD IS, which is more strongly associated with migraine with aura). The mechanisms underlying this association are unclear, but may include shared genetic determinants (see below), common features of vascular dysfunction [30], such as endothelial dysfunction [31, 32], or other yet unknown factors.

Regarding vascular risk factors, their frequency was compared between 690 patients with CeAD and age-, gender-, and country-matched patients with non-CeAD IS (N = 556), and referents from the general population (N = 1,170) [33]. Compared to referents, CeAD patients were more frequently hypertensive (in line with an independently reported association with ultrasound markers of carotid stiffness [34]), and had a lower prevalence of hypercholesterolemia, obesity and overweight. All vascular risk factors were less common in CeAD patients compared to non-CeAD IS patients. These findings suggest that atherosclerosis is probably not a major predisposing condition to CeAD [35, 36]. It has also been speculated that lean persons may have less adipose tissue protecting the arteries from minor cervical traumas, and genetic pleiotropy may play a role (see below).

Finally, CeAD shows an intriguing correlation with other non-atherosclerotic vascular diseases [37], mainly fibromuscular dysplasia (FMD) and reversible cerebral vasoconstriction syndrome (RCVS).

FMD affects primarily the renal and extracranial carotid and vertebral arteries, although other arterial beds can be involved [38, 39]. The vast majority of patients are women (~90%) with a mean age at diagnosis of approximately 52 years, and a high prevalence of hypertension [40]. In the largest published FMD registry on over 400 patients, the frequency of CeAD in FMD was 12.1% when considering initial clinical manifestations, and 19.7% when considering CeAD events that had occurred prior to enrolment in the registry [40-42]. The frequency of FMD in CeAD registries varies widely, ranging between 5.6% in a large

recent multicenter series of 983 CeAD patients [43], and 16.5-21% in older single center series on 102 [44], or 62 CeAD patients [45], (the latter two being mostly investigated with digital subtraction angiography). The mechanisms underlying the association between CeAD and FMD are unclear, and may include common genetic risk factors (see below).

RCVS is a rare, underdiagnosed cause of severe headache with segmental constriction of cerebral arteries resolving within 3 months [46], facilitated by exposure to vasoactive drugs and the post-partum period. In a recent series of 173 RCVS cases and 285 CeAD patients, 20 patients (12% of RCVS and 7% of CeAD patients) had both disorders [47]. Underlying mechanisms of RCVS are unknown and reasons for the phenotypic overlap with CeAD remain elusive.

GENETIC RISK FACTORS

In rare instances, CeAD can occur as part of a monogenic condition (either as part of a known inherited connective tissue disorder or with a Mendelian inheritance pattern but unknown causal gene). In the vast majority of cases CeAD is believed to be a multifactorial, complex disorder.

Monogenic Forms of CeAD

Seldom, CeAD can occur as a complication of known, rare inherited connective tissue disorders [48], mostly vascular Ehlers-Danlos syndrome (caused by mutations in the *COL3A1* gene) [49], but also classic or hypermobile Ehlers-Danlos syndrome (caused by mutations in *COL5A1*, *COL5A1*), Marfan syndrome (*FBN1*), Osteogenesis Imperfecta (*COL1A1*, *COL1A2*), Loeys-Dietz syndrome (*TGFBR1*, *TGFBR2*) [50, 51]. These represent less than 1/1000 of CeAD patients hospitalized in departments of neurology [50]. However, a positive diagnosis of an underlying inherited connective tissue disorder, and precise molecular diagnosis thereof, can have important implications in terms of treatment, preventive measures and follow-up, as well as genetic counselling [52]. Patients with vascular Ehlers-Danlos syndrome for instance are at increased risk of vessel rupture secondary to endovascular investigations or procedures, at high bleeding risk under anticoagulants, and celiprolol is often prescribed to prevent vascular complications [53]. Hence it is important to screen patients for clinical features and a family history suggestive of an inherited connective tissue disease (of note, family history can be negative due to a high frequency of de novo mutations). It should also be noted that currently large published series of CeAD patients have not systematically screened patients for mutations in genes causing known, inherited connective tissue disorders. Pilot data suggests that such mutations may be more common than suspected based on clinical criteria [54-59], however this raises the question of the clinical significance of these mutations and the ethical relevance of such a systematic screening, which is currently not recommended. Next generation sequencing, which enables rapid sequencing of the whole genome or coding regions at increasingly affordable cost, is changing perceptions and leading to important ethical controversies around such questions, not only in the context of CeAD [60], and improvements in reliability of sequencing and

functional annotation of variants may potentially in the future lead to changes in paradigms in some instances.

Familial cases of CeAD are rare (1-2.5% in published series) and usually do not appear to occur in the context of a known inherited connective tissue disorder [61]. Linkage analyses have not identified any significant linkage peak to date, but their power was limited [62]. Next generation sequencing may provide novel insight into the genes underlying these familial forms in the near future. An increased risk of CeAD recurrence has been described in patients with a family history of CeAD [61].

Complex Forms of CeAD

In most CeAD cases, there is no evidence for an underlying monogenic disease and CeAD seems to occur as part of a multifactorial predisposition. Heritability estimates of apparently sporadic CeAD are not available in the literature. Numerous genetic association studies based on a candidate gene approach have tested the association of CeAD with single genetic variants, on relatively small samples [62-64]. Of these, 5 have reported significant associations with variants in 3 candidate genes: *ICAM-1* (rs5498) [65], *COL3A1* (3' UTR 2-bp deletion) [58], and *MTHFR* (*MTHFR*-C677T) [66-68]. The first two were not replicated and a meta-analysis supports a modest association between the *MTHFR*-677TT genotype and CeAD [62]. However, these studies were markedly underpowered. Moreover, candidate gene association studies are unable to identify novel genetic variants involved in unsuspected pathways [69]. A large, multicenter genome-wide association study (GWAS) of CeAD was recently completed by the CADISP consortium, using DNA samples from over 2,000 CeAD patients in total [12]. GWAS consist of genotyping large numbers of genetic variants distributed across the chromosomes, and has been applied to multiple complex diseases, including stroke [70-72], in the past few years, leading to important novel insight into disease pathophysiology. This first CeAD GWAS led to the identification of common intronic genetic polymorphisms in the *PHACTR1* gene associated with CeAD risk (the lower frequency allele [G] of the top variant, rs9349379, was associated with a lower risk of CeAD) [12]. This association was confirmed in an independent sample. Other highly suggestive associations were observed, with common variants in the *LRP1* gene and the *LNK1* gene, but these require confirmation in independent samples. While the *PHACTR1* variants were equally associated with carotid and vertebral artery dissections, the *LRP1* and *LNK1* variants were associated primarily with carotid artery dissection [12]. Intriguingly, independent GWAS have found the G allele of rs9349379 to be associated with a significantly lower risk of migraine (without aura) [73, 74] and fibromuscular dysplasia [75], and significantly increased risk of myocardial infarction and coronary calcifications [76, 77]. Opposite effects of the same genetic variant on different diseases have been described elsewhere [30], suggesting either that the same region harbors different causal variants or that the same causal variant has biological effects with opposite implications for each disease. This would be consistent with the aforementioned observation from epidemiological studies that CeAD is not associated with an atherosclerotic risk profile. *PHACTR1* being a major susceptibility locus for CeAD, migraine, fibromuscular dysplasia, and coronary artery disease, understanding the mechanisms by which this locus appears to influence key vascular functions could potentially have major applications for the treatment of these conditions [12]. Interestingly, recent analyses of tissue-specific gene

expression have shown that the G allele of rs9349379 is associated with significantly reduced *PHACTR1* expression levels in coronary arteries (data in cervical arteries are lacking). *PHACTR1* is located in a highly conserved genomic region, suggesting a crucial involvement in biological processes [39], but its function is poorly understood. Recent data suggest that *PHACTR1* regulates skeletal and cardiac alpha-actin gene expression, with its expression in the heart being modulated by direct mechanical stretching of cardiomyocytes – whether this applies to mechanical stretching of cervical arteries is unknown [78]. Other data suggest that disruption of phactr-1 pathway may trigger pro-inflammatory and pro-atherogenic factors in experimental models [79].

TREATMENT AND OUTCOME

At the acute phase of ischemic stroke caused by CeAD, intravenous thrombolysis is not contra-indicated based on current evidence [80-82]. Whether it is as efficient as for other etiologies of ischemic stroke is unclear [82].

Treatment at the acute phase of CeAD (with or without cerebral or retinal ischemia) currently consists mainly of antithrombotic therapy to prevent cerebral or retinal ischemia, either by anticoagulants or by antiplatelet agents. The choice between the two is based on empirical arguments, such as presence, size and distribution of brain infarcts [83, 84]. The main rationale for using anticoagulants, a primary choice in many centers, is the observation that the mechanism of ischemic stroke in CeAD is thromboembolic in the vast majority of cases (an assumption derived from the neuroimaging distribution of ischemic lesions) [85]. However, evidence for the superiority of anticoagulants versus antiplatelet agents is lacking. Several Cochrane systematic reviews and meta-analyses of observational studies do not suggest any difference between anticoagulants and antiplatelet agents on death and disability, or on risk of cerebral infarction [86-88]. A more recent meta-analysis using a Bayesian approach found weak evidence in favor of using antiplatelet agents vs. anticoagulants, but observed associations were no longer significant after keeping only high quality studies [89]. A phase 2 randomized trial testing the feasibility of a phase 3 randomized trial comparing anticoagulants to antiplatelet agents in acute CeAD was recently published [13]. Patients with CeAD with onset of symptoms within the past 7 days, and imaging evidence of definite or probable dissection, were randomized to receive anticoagulants (vitamin K antagonists with a target international normalized ratio between 2 and 3) or antiplatelet agents (chosen at the discretion of the practitioner). The sample size required for this feasibility phase was estimated at 250, based on rates of stroke recurrence reported in observational studies. After 3 months follow-up 3 out of 126 (2%) patients under antiplatelet agents versus 1 out of 124 (1%) patients under anticoagulants sustained a recurrent stroke, which did not differ significantly (OR = 0.33 [95%CI 0.01–4.23], $p = 0.63$). No death occurred, one patients suffered major bleeding (subarachnoid hemorrhage) in the anticoagulant group. Based on the number of events in both treatment groups the authors estimate that 10,000 participants would be required for a phase 3 trial, which, given the low incidence of CeAD, would require several years of recruitment by hundreds of stroke centers. Another important observation from this pragmatic feasibility trial is that central review of imaging failed to confirm dissection in 20% of patients. Although per-protocol analyses yielded similar results this is a

demonstration of the complexity of CeAD diagnosis despite well validated and published diagnostic criteria, highlighting the importance of centralized validation of inclusion criteria in trials but also non-interventional studies on CeAD. Further information may be obtained in the near future from the ongoing treat-CAD trial, run in Switzerland, that incorporates in the outcome the presence of novel “silent” cerebrovascular lesions (ischemia on diffusion weighted imaging, micro- or macrobleeds on T2* imaging) on follow-up MRI, in addition to recurrent clinical stroke (<http://clinicaltrials.gov: NCT02046460>). This may increase the number of events, but the clinical significance of these “silent” lesions will be a point of discussion.

In case of residual arterial abnormalities (stenosis, dissecting aneurysms or occlusion), long-term antiplatelet therapy is usually prescribed. If the dissected artery is completely recanalized no long-term antithrombotic treatment is required. The decision on whether to leave patients on long-term antiplatelet therapy is usually taken after 6-12 months. Indeed, epidemiological studies have shown that the dissected artery usually “heals” within the first 6 months and radiological changes are seldom observed beyond 1 year [5].

Importantly, the rate of recurrent or de novo cerebral ischemia after treatment initiation is low in CeAD patients (<3% in the largest observational studies and the CADISS trial) [9, 13, 87, 88, 90] with most events occurring in the first weeks after diagnosis. Similarly, CeAD recurrences are also rare and seem to be most frequent within the first months (2.1% at 3 months in a series of 900 CeAD patients [9]). While mortality rates after CeAD are very low and functional outcome is usually good (~75% of CeAD patients are independent at 3 months after stroke), the psycho-social impact of CeAD can be substantial, profound fatigue and important anxiety being the most common complaints.

CONCLUSION

To summarize, several large collaborative efforts have led to important new findings on the mechanisms and treatment of CeAD, including the first large reports on phenotypic overlap between CeAD and non-atherosclerotic vascular comorbidities, the first genome-wide association study of CeAD revealing a significant association with variants in PHACTR1 showing extensive pleiotropy with other vascular conditions, and finally publication of the first randomized feasibility trial on anticoagulants vs. antiplatelet agents in CeAD.

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Chapter 39

NEW INSIGHTS IN ISCHEMIC STROKE: ROLE OF ANTIOXIDANT SUPPLEMENTATION

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ABSTRACT

Stroke causes 9% of all deaths around the world and is the second most common cause of death, after ischemic heart disease. Since the majority of stroke cases are non fatal, its major burden is long-term disability among western people. After ischemic stroke, reactive oxygen species have been implicated to play a pivotal role in brain injury. Indeed, evidence reports a rapid increase in oxidative stress-related markers production immediately after acute ischemic stroke and that antioxidant defenses are rapidly overwhelmed, thus allowing further tissue damage. At high concentrations, these free radicals react and damage the cellular macromolecules leading to cell injury and necrosis. On the other hand, lower concentrations of reactive oxygen species behave as mediators in signaling involving mitochondria, DNA repair enzymes, and transcription factors that may lead to apoptosis after cerebral ischemia. When spontaneous reperfusion occurs, rapid restoration of blood flow increases the level of tissue oxygenation, accounting for a burst of reactive oxygen species that leads to the so-called reperfusion injury. Stroke is characterized by an ischemic core (infarct) surrounded by a “penumbra” (peri-infarct) region. In the penumbra area, where collateral blood flow can buffer the effects of vessel occlusion, cell death occurs less rapidly via oxidative stress-mediated mechanisms such as apoptosis and inflammation and thus, arises as an emerging target for therapeutic treatment. The currently applied measures to protect the brain against the severe damage caused by stroke have yielded insufficient improvement in the outcome of these patients. Thus, antioxidant strategies that lead to diminution of the oxidative injury are key to investigating new treatments to overcome the consequences of stroke. Evidence on acute

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antioxidant system enhancement by means of use of NADPH oxidase inhibitor apocynin, xanthine oxidase inhibitor allopurinol, antioxidant vitamins C and E, the polyphenol resveratrol, as well as other antioxidant strategies are reviewed in the setting of stroke. Therefore, we introduce new strategies to be tested to reduce the cerebral damage related to both ischemia and reperfusion. This chapter reviews the mechanisms involved in ROS generation, the role of oxidative stress in the pathogenesis of ischemic stroke, the current strategies to treat ischemic stroke and new possible therapeutic strategies to treat this disease.

Keywords: Stroke, oxidative stress, antioxidant defense system

ABBREVIATIONS

BBB:	blood-brain barrier
CAT:	catalase
CNS:	central nervous system
COX:	cyclooxygenase
GSH:	glutathione
GSH-Px:	glutathione peroxidase
HPLC:	high performance liquid chromatography
MCA:	middle cerebral artery
MDA:	malondialdehyde
MMP:	matrix metalloproteinase
NAC:	N-acetylcysteine
NF- κ B:	nuclear factor kappa B
NO:	nitric oxide
NOS:	nitric oxide synthase
OGD:	oxygen glucose deprivation
ROS:	reactive oxygen species
rtPA:	recombinant tissue plasminogen activator
SCU:	stroke care units
SOD:	superoxide dismutase
TBARS:	thiobarbituric acid reactant substances
TIA:	transient ischemic attack
VEGF:	vascular endothelial growth factor

INTRODUCTION

Stroke is the third most common cause of death in the population [1, 2]. Since life expectancy continues to grow in developed countries, the absolute number of individuals suffering from stroke will further increase in the near future [3]. The mortality rate in the acute phase of stroke is as high as 20% [1] and remains higher for several years after the acute event compared to general population [4].

The majority of strokes are non fatal, and the major burden is long-term disability. Therefore, it constitutes the most important single cause of severe disability among western people [5]. Because of the burgeoning elderly population in western societies, is estimated that by 2030 stroke-related disability in western societies will be ranked as the fourth most important cause of reduced disability-adjusted life-years (DALYs - the sum of life-years lost as a result of premature death and years lived with disability adjusted for severity) [6].

Stroke can be subdivided into 2 categories, ischemic and hemorrhagic. Ischemic strokes are more prevalent than hemorrhagic, making up approximately 87% of all cases, and being the target of most drug trials [7]. Classification based on the TOAST system [8] identifies the mechanism that leads to vessel occlusion (cardioembolic, artery to artery embolism, or in-situ small-vessel [lacunar] disease) and is important in the patient management because such information should influence both acute treatments and secondary prevention strategies [9]. A significant proportion of ischemic strokes is due to atherothrombosis which is related to several conditions associated with increased oxidative stress, such as hypertension, diabetes, dyslipidemia [10, 11].

RISK FACTORS

Aging is one of the most important risk factors for developing stroke. In fact, the majority of patients who experience stroke are older than 65 years [3]. Since a positive correlation between oxidative stress and aging has been reported [12], increased age could contribute to the occurrence of oxidative stress in the setting of stroke. There is a continuous association between both systolic and diastolic blood pressures and the risk of ischemic stroke [13]. Meta-analyses of randomized controlled trials confirm an approximate 30% to 40% stroke risk [14]. Diabetes is also a clear risk factor for stroke [15]. Hypercholesterolemia and hyperlipidemia are not as well established as risk factors for first or recurrent stroke in contrast to what is seen in cardiac disease [16]. In contrast, there is strong and convincing evidence that cigarette smoking is a major independent risk factor for ischemic stroke [17]. Obesity, defined as a body mass index of 30 kg/m², has been established as an independent risk factor for coronary heart disease and premature mortality [18]. The risk associated with smoking is present at all ages, in both sexes, and among different racial/ethnic groups [19]. In the other hand, most brain damage caused by stroke is a consequence of inflammation. Accordingly, plasma C-reactive protein levels have demonstrated to offer a valuable biomarker for tracking mortality from acute stroke, as well as risk of recurrent stroke [20].

Since a positive correlation between oxidative stress and all of this major risk factor has been reported, increased age, diabetes, obesity, hypertension and smoking could contribute to the worse evolution in the acute stroke event [12, 21-23]. Patients with these risk factors may have a lower antioxidant defenses functional reserve to cope with the stroke acute event.

Hypercholesterolemia and hyperlipidemia are not as well established as risk factors for first or recurrent stroke in contrast to what is seen in cardiac disease [16]. Monitoring lipid profile remains valuable; despite half of all strokes in the United States occur in people who do not have abnormal cholesterol or triglyceride levels [24]. In addition, multiple epidemiological studies have reported that high plasma homocysteine levels associate with increased risk for stroke [25]. Indeed, elevated plasma total homocysteine is associated with oxidative stress, hypercoagulability, increased cholesterol synthesis, abnormally reduced and dysfunctional

HDL-cholesterol, and endothelial dysfunction [26-30]. The effect of alcohol on stroke risk is controversial; however there is strong evidence that chronic alcoholism and heavy drinking are risk factors for all stroke subtypes [31].

PROTECTIVE FACTORS

Fruit and vegetable consumption has been associated with lower risk of stroke [32], which may be related to their antioxidant properties [21, 33]. Substantial evidence exists that physical activity exerts a beneficial effect on multiple cardiovascular disease risk factors, including those for stroke [34-36].

It is known that folate is the major metabolic recycler of homocysteine to methionine [37]. A recently published meta-analysis concluded that folate is effective in preventing stroke [38]. In the Heart Outcomes Prevention Evaluation 2 study, the combination of folic acid, vitamin B6, and vitamin B12 versus placebo for an average of five years demonstrated a significant stroke risk reduction [39].

PATHOPHYSIOLOGY

Oxidative Stress

Oxidative stress constitutes a unifying mechanism of injury of many types of disease processes, it occurs when there is a serious imbalance between the generation of ROS and the antioxidant defense system in the body so that the latter become overwhelmed [40].

ROS are a family of highly reactive species that are formed either enzymatically or non-enzymatically in mammalian cells. Superoxide ($O_2^{\cdot-}$) disproportionates quickly to oxygen and hydrogen peroxide (H_2O_2). In addition, various cellular oxidases, such as monoamine and amino acid oxidases, generate H_2O_2 . In the presence of redox-active iron, highly reactive and toxic hydroxyl radicals are generated from H_2O_2 and superoxide in the Fenton reaction and the Haber–Weiss cycle [41].

ROS are implied in both physiological and pathophysiological processes. For example, ROS play a beneficial role in the immune system by activating T lymphocytes and IL-2 production and mediating neutrophils and macrophages phagocytosis, participate in oxygen homeostasis, secondary messenger system and signal transduction [42- 44]. Particularly in CNS reactive oxygen species are mainly generated by microglia and astrocytes and modulate synaptic transmission and nonsynaptic communication between neurons and glia. In addition to these roles, ROS can induce synaptic longterm potentiation required for memory formation and evidence suggests that there is an age-dependent alteration in the role of $O_2^{\cdot-}$ in modulating synaptic plasticity, learning, and memory formation [45].

Superoxide is one of the most important ROS in CNS, but besides its above-mentioned role, it has also the potential to harm the ROS-producing cell, as well as neighboring cells. Superoxide is generated as a by-product of the respiratory chain or as a product of enzymes such as xanthine oxidase or NADPH oxidase. Although xanthine oxidase was identified early as a ROS source during neuronal reperfusion, other intracellular sources particularly NADPH

oxidase have been shown to produce ROS and contribute to oxidative stress during reperfusion [46]. NADPH oxidase type 4 (NOX4) is the most abundant vascular isoform; its expression is even higher in cerebral than in peripheral blood vessels [14] and, further, induced in stroke [15]. Therefore, it has been hypothesized that NOX4 is the most relevant source of ROS in stroke.

To avoid ROS-mediated cellular damage, such as lipid peroxidation, protein carbonilation, and DNA strand breaks, antioxidative mechanisms are present in cells that remove ROS or even prevent their generation. Low-molecular-weight antioxidants such as glutathione (GSH), vitamin C and vitamin E contribute to the antioxidant potential of cells. In addition, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidases (GSH-Px), and glutathione reductase (GR) are important for the cellular defense against ROS. H₂O₂ generated as a product of SODs is substrate of the heme-containing catalase that converts H₂O₂ to oxygen and water. In addition, GSH-Px uses GSH to reduce H₂O₂ to water.

Because of the short half-life of ROS, direct measurement within the brain is difficult in human subjects. The product formed by ROS attack to biomolecules constitutes a useful tool for oxidative stress assessment. Beyond the initial damage to membranes, reaction of these radicals with double bonds of fatty acids in lipids produces peroxides that give rise to α,β -unsaturated aldehydes including malondialdehyde (MDA), 4-hydroxynonenal and acrolein [47].

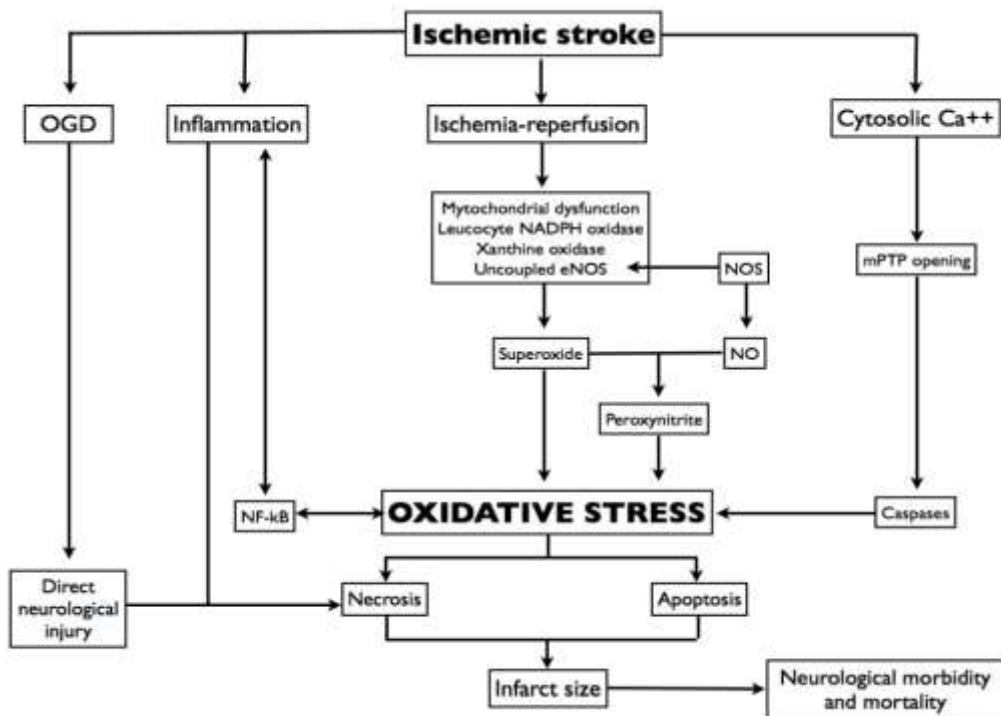


Figure 1. Role of oxidative stress in the pathophysiology of neuron injury after ischemic stroke, leading to infarct size and neurological morbidity and mortality.

Unlike other organs the brain is especially vulnerable to ROS due to low neuron antioxidant enzyme levels (catalase, glutathione peroxidase) and high concentrations of peroxidisable lipids, high oxygen consumption, and high levels of iron that act as pro-oxidants under pathological conditions [48]. In agreement with this view, free radical production has been reported as an important mechanism of brain injury after exposure to ischemia and reperfusion [49].

ISCHEMIA – REPERFUSION

Brain injury following stroke results from the effects of blood flow restriction caused by alterations such as thrombosis, embolism or systemic hypo-perfusion, basically resulting in cellular hypoxia and glucose deprivation. Ischemia initiates a complex cascade of metabolic events, several of which involve the generation of nitrogen and oxygen free radicals. These free radicals and related reactive species mediate most damage that occurs after transient brain ischemia in the penumbral region of infarcts [50] (figure 1). The pathological process involved after cerebral ischemic reperfusion injury are mainly oxidative stress, excitotoxicity, intracellular calcium overload, as well as inflammation and apoptosis [51].

The sequence of events begins at the mitochondrial level with a critical diminution in electron transport chain activity with the consequent ATP depletion [52]. Sequentially, once mitochondrial ATP synthesis has been inhibited by global ischemia, ATP consumption takes place within 2 minutes, causing neuronal plasma membrane depolarization, potassium leakage to the extracellular space and entry of sodium into cells [53]. This bioenergetic impairment also prevents the plasma membrane Ca^{2+} -ATPase from maintaining the normally low cytosolic calcium concentration.

Rapidly following cessation of blood flow, and the compromised energy metabolism quickly leads to large increases in neuronal activity and a resulting enhancement in glutamate release [54]. Neuronal release of glutamate following cerebral ischemia activates several types of pre- and post-synaptic glutamate receptors with the consequent rise in intracellular calcium concentration, determining an excitotoxic injury. High levels of intracellular Ca^{2+} , Na^{+} and ADP may lead to mitochondrial dysfunction, generation of reactive oxygen species, and the activation of proteases, phospholipases, and endonucleases, leading to cell death [55].

In addition, elevated extracellular glutamate concentrations can activate neuronal NOS via calcium influx [56]. As the level of superoxide ion is also elevated [57], the NO radical has dual effects: (1) to combine with superoxide anion to form highly pro-oxidant peroxynitrite and (2) to interfere with superoxide dismutase by reducing its antioxidant effect [58, 59].

Three distinct phases of ROS generation have been identified in cellular cultures, occurring with a specific temporal relationship to metabolic events taking place within the cells [60]. The first increase in ROS production during oxygen and glucose deprivation (OGD) coincide with the period of mitochondrial depolarization and that significant ROS generation ceased at the time that mitochondrial potential was completely collapsed. Inhibition of mitochondrial respiration by OGD inhibits cell respiration at complex IV, causes accumulation of reduced intermediates of the respiratory chain, and allows electron leakage to generate ROS [60]. The inhibition of the secondary increase of ROS production (after 25–35

min OGD or chemical ischemia) by xanthine oxidase inhibitors [60], strongly suggests a contribution of the enzyme in this response. It has been previously suggested that depletion of intracellular ATP leads to conversion of adenine nucleotides to hypoxanthine and xanthine, substrates for xanthine oxidase [61, 62].

It has been described that early reperfusion preserves penumbral brain tissue whereas late reperfusion increases ischemic damage [63]. Rapid restoration of blood flow increases the level of tissue oxygenation, leading to a third phase of ROS generation that causes the so-called reperfusion injury [64]. This phase can be blocked by NADPH oxidase inhibitors and was absent in cells from a NADPH oxidase – knockout (gp91^{phox} -/-) mice [60]. Also, activation of NADPH oxidase through Ca²⁺-dependent pathway was partly attributable to glutamate release and excitotoxicity. Furthermore, microdialysis of the striatum using sodium salicylate, which reacts with OH⁻ showed that this radical is formed mainly after reperfusion [65, 66]. Another study revealed that ROS generation during reperfusion was shown to be mainly in the endothelium and smooth muscle cells of small blood vessels [67], being likely to contribute to BBB disruption in vivo models.

We must highlight that in stroke patients another mechanisms contribute to the generation of free radicals. A major source of ROS in stroke patients are infiltrating neutrophils, microglia / macrophages and endothelial cells, accounting for another rise in ROS levels. Additionally, elimination of NOX4 remained beneficial in the absence of arterial recanalization in humans [68], suggesting that NADPH oxidase also participates before the reperfusion.

Histologically, stroke is characterized by an ischemic core (infarct) surrounded by a “penumbra” (peri-infarct) region. Within the core of the ischemic area, where blood flow is most severely restricted, excitotoxic and necrotic cell death occurs within minutes. In the penumbra area, where collateral blood flow can buffer the full effects of the stroke, cell death occurs less rapidly via oxidative stress-mediated mechanisms such as apoptosis and inflammation [69] and is an important target for therapeutic intervention. Cellular death mechanisms of penumbra are detailed below.

APOPTOSIS

The mode of cell death that prevails probably depends on the severity and precise nature of the ischemic injury. However, accumulating findings demonstrate that p53 is involved in neuronal death both in vitro hypoxic conditions [70, 71] and in vivo cerebral ischemic animal models [70, 72-74]. Moreover, levels of p53 and its products increase during early hours after ischemia onset and are especially high in the infarct border zone in focal cerebral ischemia models [75, 76], suggesting a key role in cellular death at the penumbra area.

Studies have emphasized the role of peroxynitrite in causing single-strand breaks in DNA, which activates DNA-dependent kinase and ataxia telangiectia protein, leading to activation, stabilization, and up-regulation of p53 [77-79]. The phosphorylation of p53 by stress-induced kinases reduces the affinity of p53 for MDM2 and enables a tighter association between p53 and its coactivators, such as the histone acetyl transferase (HAT) p300, increasing specific DNA binding at target genes.

INFLAMMATION

Interruption of blood flow to the brain leads to energy loss and necrotic cell death in the ischemic core. This initiates the immune response, characterized by a rapid activation of resident microglial cells and astrocytes, and by neutrophils and macrophages infiltration into the brain as demonstrated both in animal models and in stroke patients [80-84].

Four to six hours after ischemia, astrocytes become hypertrophic, followed by activation of microglial cells that evolve into an ameboid type with an enlarged cell body and shortened cellular processes [85-87]. In the other hand, within hours after the ischemic insult, increased levels of cytokines and chemokines enhance the expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) which reach a peak between 6 and 12 h after the onset of ischemia [88]. The increased expression of adhesion molecules can promote the adhesion and transendothelial migration of circulating monocytes and neutrophils.

Infiltrating neutrophils, microglia / macrophages and endothelial cells may release toxic amounts of NO mainly via the inducible NOS isoform, which is strongly induced following the ischemic injury both in animal models [89, 90] and in stroke patients [91] and also through activation of neuronal NOS [92]. NADPH oxidase expressed on the plasma membrane of the microglia can additionally contribute to the burst of ROS generation [93, 94].

During the first 2 hours after brain ischemia, NO produced by endothelial NOS exerts beneficial effects by promoting vasodilatation, decreasing platelet aggregation and leukocyte adhesion to the endothelium. Nevertheless, NO produced during later stages can rapidly interact with superoxide to form peroxynitrite, the key ROS in ischemic brain injury [95-97]. Furthermore, NO is responsible for cytotoxicity by inhibiting ATP-producing enzymes, by producing peroxynitrite and by stimulating other pro-inflammatory enzymes such as COX-2 [98].

As well as inflammatory cells are capable to produce ROS, ROS are capable to activate inflammatory cells via several mechanisms, determining a vicious circle. These mechanisms include ROS activation of NF- κ B, which is a highly redox-sensitive transcription factor mediating the activation of microglia, neutrophils and macrophages, and locally, ROS induction of the astrocyte expression of cytokines mediates activation of microglia [99].

Increasing evidence indicates that brain ischemia can also cause significant changes in the peripheral immune system [100-102]. Stroke causes neutrophilia, lymphocytopenia and an increase in the number of circulating monocytes [103]. However, although the increase in neutrophil and monocyte number is likely to contribute to ischemic damage, this change is also evident in patients that have experienced a transient ischemic attack [103], suggesting that infiltration to the brain would be the key determinant of whether these changes contribute to ischemic injury.

BLOOD BRAIN BARRIER DISRUPTION

It has been largely confirmed in cell culture models that ROS have a key role increasing permeability of the cerebral endothelium through the formation of actin stress fibers and migration of ZO-1 and occludin, accounting for the BBB disruption [104, 105].

There are two phases in BBB disruption. Early disruption of the BBB is likely to be primarily mediated by altered endothelial cell function. It has been reported that inhibition of the NADPH oxidase with apocynin was effective in maintaining early BBB function. Genetic deletion of gp91phox was also effective preventing early BBB dysfunction and offered protection from brain swelling after stroke, suggesting an important contribution of NADPH oxidase in early BBB disruption. Cellular origin of NADPH oxidase (i.e., leukocytes, microglia, neurons or endothelium) still remains unclear [106].

Late disruption is a consequence of inflammation, cell necrosis, production of tissue-degrading enzymes such as matrix metalloproteinases, and alterations in gene expression; therefore, late BBB disruption represents a very complex context [107-109]. ROS also participate in the late disruption, through matrix metalloproteases activation, degrading collagen and laminin in the basal lamina, which disrupts the integrity of the vascular wall and increases blood brain barrier (BBB) permeability. NO seems to be the most important molecule in terms of MMP interactions and it has been suggested to regulate MMPs via several routes. NO can directly activate MMP-9 by reacting with cystine residues in the propeptide domain of MMP-9 to form S-nitrosylated derivatives [110]. In addition, NO upregulates MMP-9 by increasing MMP-9 mRNA turnover [111].

The greatest significance of BBB disruption is that it results in tissue swelling and so increase regional tissue hydrostatic pressure. Thus it is possible that the primary disturbance of the barrier properties of the cerebral endothelium, leading to local tissue edema, will impose a constriction on the compliant vascular structures (i.e., the veins), resulting in reduced perfusion [112].

EDEMA FORMATION

Brain edema is a leading cause of death after stroke. Free radicals exert their deleterious actions leading to both cytotoxic and vasogenic edema, as it will be detailed below. Cytotoxic edema, which is defined as a swelling of cellular components in the brain, is the primary mechanism responsible for brain edema during the earliest phase of ischemic injury and results from cellular metabolic disturbances. All cellular elements (neurons, glia, and endothelial cells) take in fluid and swell but do not contribute to a net increase in brain volume because a corresponding reduction in the extracellular fluid space also occurs. Cytotoxic edema depends primarily on the duration and depth of ischemia and is an important indicator of ultimate infarct size and stroke severity.

The perturbation of ion transport perhaps plays the most important role in the pathophysiology of cellular swelling. It has been proposed an ion transporter modulation by ROS, including the peroxidation of membrane phospholipids, the oxidation of sulfadyl groups in ion transporters, and protein modification [113]. Indeed, iron-generated free radicals in vitro inhibit Na⁺, K⁺ -ATPase, Ca²⁺ -ATPase, calmodulin-associated Ca²⁺ -ATPase, and Na⁺ -Ca²⁺ exchanger activities [114].

Oxidative stress also supports the formation of cytotoxic edema through interaction with others mediators of cellular swelling. For example, lacticidosis is known to cause swelling of neurons and astrocytes triggering the release of iron from its binding site, which increases hydroxyl radical formation by increasing intracellular hydrogen peroxide levels and by

supplying protons during the chain reaction between superoxide and nitric oxide (NO) [115]. In addition, one study showed the potential contribution of ROS to AQP2 dysfunction in ischemia reperfusion-induced renal injury in a rat model [116].

In the other hand, vasogenic edema is caused by uncontrolled fluid leakage from the blood to the brain parenchyma through a weakened BBB and contributes to an actual net volume increase of the brain. Vasogenic edema is usually formed later than cytotoxic edema, after the cessation of cerebral blood flow; however, the disruption of the BBB can be demonstrated as early as 20 min after transient global forebrain ischemia.

As discussed above, free radicals such as superoxide and NO exert direct effects on mediators of BBB disruption such as leukocytes, inflammatory response, MMP and microvascular integrity. ROS also contributes to VEGF expression, leading to vasogenic edema. Moreover, a vascular NADPH oxidase, a main source of vascular superoxide, is involved in VEGF expression [117].

OXIDATIVE STRESS BIOMARKERS

Finally, it must be emphasized that stroke is an important factor in the overall antioxidant capacity and ROS production [118, 119].

The size and type of the stroke is an important factor in the overall antioxidant capacity and oxidative stress. Several studies described increased levels of lipid peroxidation markers in stroke patients, such as MDA assessed by TBARS [120-122] and by HPLC [123, 124], and lipid peroxides [120, 125], in plasma and erythrocytes.

Plasma levels of various nonenzymatic endogenous antioxidant systems, such as vitamin C [118, 122, 123], vitamin E [121], vitamin A, and uric acid [118], were also reported to be lower in patients with ischemic stroke than in a healthy control population.

These data suggest that either the aforementioned antioxidants are consumed or oxidized during the cerebral ischemia reperfusion event or that their levels are lower already before the stroke.

Data regarding the activity of some antioxidant enzymes are rather contradictory. Some studies observed an augmentation of SOD concentration in plasma [126] or cerebrospinal fluid (CSF) [126, 127], while others measured a decrease in plasma [118, 128] or erythrocytes [124] or even no modifications in erythrocytes [120]. Reduced GSH-Px activity was demonstrated in erythrocytes [124], whereas this activity appeared increased in total blood [120].

These oxidative stress markers also seem to be useful as prognostic predictors of stroke outcome. Several studies has shown that brain damage and post-ischemic neurological deficit are correlated with concentrations of SOD 1 and 3 in CSF [127], lipid peroxides in plasma [125], MDA by HPLC and MDA by TBARS in erythrocytes [120, 124], and also the extracellular production of free radicals by peripheral phagocytes [120]. A significant positive association between plasma lipid hydroperoxide concentration and the National Institute of Health Stroke Scale has been reported and a significant negative correlation with the Glasgow Coma Scale [125].

On the contrary, histological and neurological parameters have been demonstrated to be negatively correlated with plasma levels of vitamins C, vitamin E [129], and uric acid [130],

plasma total antioxidant activity [129], SOD activity in plasma [128] or in erythrocytes [124], and GSH-Px activity in erythrocytes [124].

NON-ANTIOXIDANT ACUTE INTERVENTIONS FOR STROKE MANAGEMENT

Stroke Care Units (SCU)

One of the most substantial advances in stroke has been the routine management of patients in stroke care units (SCU), which is effective and appropriate for all stroke subtypes, and provides a focus for professionals in stroke care. Management of patients within an SCU reduces mortality by about 20% and improves functional outcome by about the same amount [131]. A physical space identified as an SCU is associated with better outcomes than seen with a dedicated stroke team, visiting patients on general medical wards [132]. The important characteristics of stroke unit care within the randomized trials were the provision of coordinated multidisciplinary rehabilitation, staff specialisation in stroke or rehabilitation, and improved education and training [133]. These nonspecific actions of neuroprotection are also in the context of improving the cellular function, oxygenation and thus decreasing the oxidative stress. It has been reported that SCU was associated with better long-term survival, but younger patients, patients with intracerebral haemorrhage and patients who were unconscious had the best relative effect and may be given the highest priority to this form of care [134].

Revascularization Techniques in the Acute care

Intravenous Thrombolysis

Existing therapeutic interventions to reduce infarct size are limited to thrombolysis [135]. Thrombolysis is the only intervention currently approved by the U.S. Food and Drug Administration (FDA); it does not work effectively for many patients, being effective if applied within 4.5 hours of symptom onset [136]. In fact, in the US, only roughly 2% of stroke patients benefit from access to early thrombolysis [137]. Due to the strict time constraints for intervention, inadequate readiness at some hospitals, substantial risk of intracerebral hemorrhage, restrictive patient selection criteria, and other factors such as transport to the treatment facility, only one to three percent of stroke patients receive rtPA treatment. The major risk from rtPA intervention is hemorrhage, being its incidence around six percent [138].

Intra-arterial Thrombolysis

PROACT II is the first randomized multicenter trial to demonstrate the clinical efficacy of intra-arterial thrombolysis in patients with acute stroke of less than 6 hours' duration caused by MCA (middle cerebral artery) occlusion [139]. Direct intra-arterial delivery of fibrinolytic agents is reportedly more effective to recanalize major symptomatic cerebral arterial occlusions than intravenous delivery [140]. Combination therapy of intravenous and

intraarterial infusion of recombinant tissue plasminogen activator (rtPA) has benefits in patients with acute major cerebral artery ischemia [141].

Mechanical Thrombectomy

Endovascular mechanical thrombectomy may be used during acute ischemic stroke due to large vessel intracranial occlusion. Endovascular mechanical thrombectomy can restore vascular patency of carotid terminus or basilar artery occlusions between 41% and 54% of the time, providing an alternative or synergistic method to restore blood flow [142].

Anticoagulant and Antiplatelet Drugs

The current stroke guidelines accepts interventions to diminish platelet aggregation can reduce the risk for recurrent stroke in patients with an acute episode, recommending aspirin as the single antiplatelet drug adequately validated for this purpose. Aspirin is classified as adjunctive therapy to rtPA, and should not be started earlier than 24 hours after the onset of stroke. In patients who do not receive thrombolysis, aspirin is started from the diagnosis [143]. Clopidogrel has been the most common therapy received in the long-term care by these patients and still requires further research [144]. Treatment with GPIIb/IIIa antagonists is associated with increased mortality [145]. Warfarin remains one of the most biologically effective secondary prevention strategies for patients with atrial fibrillation and reduces the relative risk of recurrent stroke in patients with TIA or minor stroke by about 70% [9]. In the secondary prevention, the use of Aspirin plus extended-release dipyridamole is much better than the use of Aspirin alone [146].

Non-pharmacologic Neuroprotection

Airway, Ventilatory Support and Oxygen

Airway support and ventilatory assistance are recommended for the treatment of patients with acute stroke who have decreased consciousness or who have bulbar dysfunction causing compromise of the airway [147]. Hypoxic patients with stroke should receive supplemental oxygen [148].

Temperature Management

Increased body temperature in the setting of acute ischemic stroke is associated with poor neurological outcome (increased risk of morbidity and mortality), possibly secondary to increased free radical production, increased metabolic demands, and enhanced release of neurotransmitters [149]. It is generally agreed that sources of fever should be treated and antipyretic medications should be administered to lower temperature in febrile patients with stroke [150].

Blood Pressure

The management of arterial hypertension remains controversial. Both elevated and low blood pressures are associated with poor outcome after stroke [151]. It is generally agreed

that patients with markedly elevated blood pressure may have their blood pressure lowered. In the other hand, the cause of arterial hypotension in the setting of acute stroke should be sought. Hypovolemia should be corrected with normal saline, and cardiac arrhythmias that might be reducing cardiac output should be corrected [143].

Glucose Management

It is generally agreed that hypoglycemia should be treated in patients with acute ischemic stroke. The goal is to achieve normoglycemia. Marked elevation of blood glucose levels should be avoided [152].

Pharmacological Neuroprotection

Albumin

In several animal models of ischemic stroke, albumin infusion reduced infarct size and swelling, improved local perfusion to zones of critical blood flow reduction, and improved neurological and behavioral functions [153]. In a clinical trial conducted by Shin et al. [154], patients with moderate to severe ischemic stroke in the area of the middle cerebral artery were randomized within 12 hours after symptom onset. Although infarct volume continued to increase in control and albumin groups, when measured at 72-96 hours after onset, the volume increase was significantly less in the albumin-treated patient group as a whole (26.0% increase versus 67.2% increase for albumin and control groups, respectively). This protein has a prolonged circulating half-life (about 20 days), and its high molecular weight keeps it in the blood vessel lumen, drawing in water and thereby expanding intravascular volume [154]. Albumin is also a potent antioxidant, a large molecule carrying a high density of cysteine groups [155]. Albumin clearly has strong benefit in acute ischemic stroke, without troublesome adverse effects. Besides having the advantage that it can be applied anytime within 24 hours of stroke onset, as shown in the Shin trial [156], albumin is cost-effective compared to other in-hospital interventions.

Magnesium Sulfate

Ionized magnesium has demonstrated effective neuroprotection in animal models of cerebral ischemia, excitotoxic injury, head trauma, and spinal cord damage [157]. The international Intravenous Magnesium Efficacy in Stroke (IMAGES) trial suggests that magnesium has neuroprotective potential for acute ischemic stroke [158]. Although it found no statistically significant overall benefit, it did show a trend toward protection for patients with noncortical stroke [158]. FAST-MAG is a large, controlled trial with the statistical power to detect modest treatment effects of intravenous magnesium sulfate for very early stroke; this trial is in progress. This unique trial hopes to demonstrate pre-hospital initiation whether magnesium infusion therapy, mostly via paramedics, can be effective in halting or slowing the ischemic cascade in most patients within those first crucial two hours.

Hypothermia

Hypothermia slows cerebral metabolism and protects neurons subjected to acute ischemia. This is a potent intervention that improves neurological outcomes after cardiac

arrest and has been used to treat patients with severe brain edema [159]. Because the optimal body temperature for stroke management is not yet clear, caution must be taken since excessive cooling could precipitate adverse effects such as hypotension, cardiac arrhythmias, or infections [142]. Furthermore, after applying hypothermia and intravenous magnesium in animal experiments, Meloni et al. [160] suggest concomitant use of both interventions in patients should improve the overall neuroprotective outcome.

Other Pharmacological Strategies

After many promising animal studies, a human pilot study – the Goettingen EPO-Stroke Study – examined erythropoietin (EPO) for stroke [161]. EPO given intravenously readily crossed the blood-brain barrier. It was proven safe and demonstrated significantly better outcomes compared to placebo. Thus, EPO patients experienced significantly less neurological deficit, better restitution of brain function, and significantly smaller lesion size than controls.

Lubeluzole was tested in several clinical trials [162] Although a pilot study suggested that the agent was safe and might reduce the death rate, subsequent larger clinical trials found no effects in reducing deaths or improving outcomes after stroke [163].

Citicoline is an agent that apparently stabilizes membranes, has been tested in several clinical studies [164]. The trials did not demonstrate efficacy from treatment. A subsequent meta-analysis reported that patients with moderate to severe stroke might be helped if the medication was started within 24 hours of onset of symptoms [165]. This finding should not be considered definitive but rather a rationale for further testing of the medication in this subgroup of patients.

Piracetam also has been tested in several clinical trials, with mixed results [166, 167]. Although the agent may be effective in some patients with ischemic stroke, the data are not sufficiently clear to draw a conclusion about the utility of this medication.

There was no indication that careful blood-pressure lowering treatment with the angiotensin-receptor blocker candesartan is beneficial in patients with acute stroke and raised blood pressure. If anything, the evidence suggested a harmful effect [168].

THE ROLE OF ACUTE ANTIOXIDANT DEFENSE SYSTEM ENHANCEMENT IN ISCHEMIC STROKE TREATMENT

There is a substantial need for further research on agents able to significantly reduce the cerebral damage related to both ischemia and reperfusion. These agents might be particularly important not only in those patients who cannot receive thrombolysis but also in those who, undergoing this type of treatment, are at risk of the so-called reperfusion injury. Antioxidants have been evaluated as neuroprotective agents in stroke [169] since there is evidence supporting the occurrence of oxidative stress during brain ischemia. The therapeutic use of most studied antioxidants against stroke-induced brain damage is discussed below. A summary of the proposed antioxidant strategies can be found on figure 2.

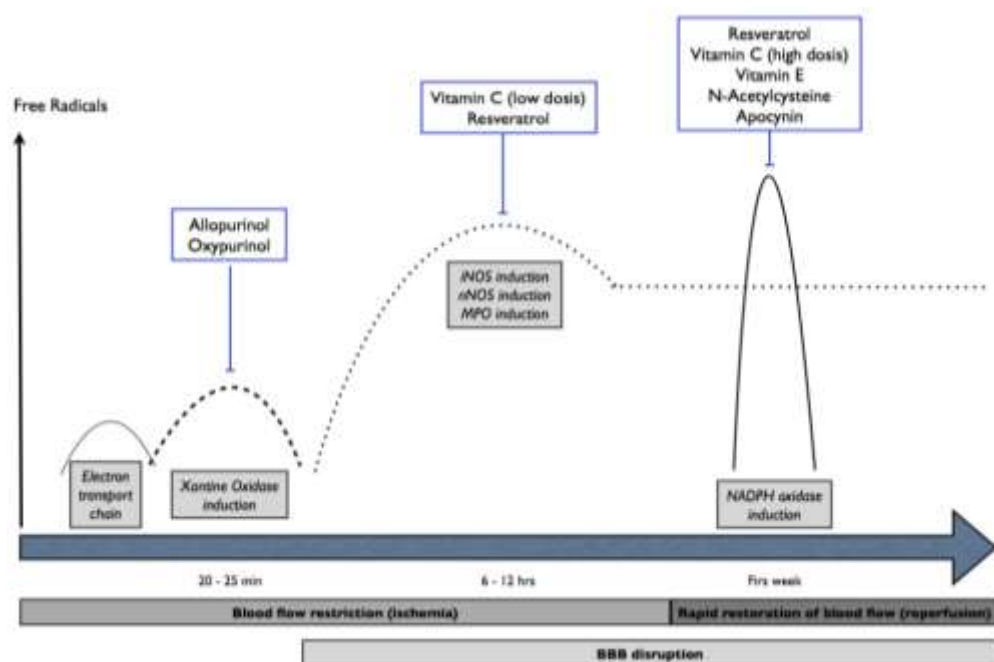


Figure 2. Therapeutic opportunities along the time course of oxidative stress development in the setting of ischemic stroke.

Inhibition of ROS-producing Enzymes

NADPH Oxidase Inhibition

Superoxide producing NADPH oxidase has been shown to contribute to oxidative stress-mediated brain injury during both ischemia and reperfusion. Apocynin (4-hydroxy-3-methoxyacetophenone, trivial names: apocynin, acetovanillone) has been used as an efficient inhibitor of the complex NADPH oxidase in many experimental models [170, 171]. The mechanism of inhibition has not yet been fully elucidated. In leukocytes, apocynin is a prodrug that is activated by myeloperoxidase, a process that results in the formation of apocynin dimers [172], which have been shown to be more efficient than apocynin itself [173, 174]. Endothelial cells and smooth muscle cells failed to form these dimers and, therefore, are not able to activate apocynin [172]. It has been demonstrated that treatment with apocynin can attenuate brain injury after experimental ischemic stroke in the hippocampus after global cerebral ischemia [175-177].

In a recent study, treatment of apocynin 5 minutes before reperfusion attenuated cerebral injury in a 24-hour ischemia model in rats [178]. Administration of apocynin showed marked reduction in infarct size compared with that of control rats. Medial carotid artery occlusion-induced cerebral ischemia was also associated with an increase in, nitrotyrosine formation, as well as IL-1 β expression, I κ B degradation and ICAM expression in ischemic regions. These expressions were markedly inhibited by the treatment of apocynin. It was also demonstrated that apocynin reduces levels of apoptosis (TUNEL, Bax and Bcl-2 expression) resulting in a reduction in the infarct volume in ischemia-reperfusion brain injury [178].

It has been demonstrated that pharmacological inhibition of NADPH oxidases using the specific NADPH oxidase inhibitor Vasopharm (VAS2870) [179-182] protects mice from brain ischemia within a clinically meaningful 2-h time window [68]. This protection resulted in decreased infarct volume, reduced oxidative stress, neuronal apoptosis, and blood-brain-barrier leakage and increased neurological score, motor function and survival.

In contrast, commonly used apocynin may not be a NADPH oxidase inhibitor in vascular cells but rather acts as a nonspecific antioxidant [172], also inhibiting Rho kinase inhibitor [183], an activity that increases its nonspecific actions. If apocynin inhibits NADPH oxidases at all, it supposedly blocks the migration of the cellular NADPH oxidase complex subunit p47phox to the membrane, thus interfering with assembly of the functional NADPH oxidase complex [184]. Therefore, it is unlikely to inhibit the NOX4-containing NADPH oxidase, which acts independently of any cytosolic subunits [185]. Indeed, application of apocynin had no effect on the formation of ROS or of functional outcome after experimental stroke in vivo in a NOX4 *knock out* model [68].

Allopurinol

Ischemia-induced depletion of intracellular ATP leads to accumulation of hypoxanthine and xanthine, substrates for ROS-producing xanthine oxidase which is one of the main sources of ROS in the setting of ischemia-reperfusion [186]. Allopurinol is an inhibitor of the xanthine oxidase enzyme; furthermore, with its metabolite oxypurinol have been suggested to scavenge hydroxyl radical [187]. In addition, evidence suggests that hypoxic and reperfusion injury to the cerebral vasculature, especially to the endothelial cells, is dependent on xanthine oxidase [188, 189].

Allopurinol is an inhibitor of the xanthine oxidase enzyme, which is thought to be the main source of ROS in the setting of ischemia-reperfusion [186]. Among the molecules preventing the formation of free radicals, xanthine oxidase inhibitors, such as allopurinol or its metabolite oxypurinol, reduced cerebral infarct size and edema in permanent ischemia [190-193].

Allopurinol was found to be protective against mortality and neurological impairment in a stroke model in spontaneously hypertensive rats [194]. In a permanent middle cerebral artery occlusion model in Sprague-Dawley rats, allopurinol pretreatment reduced infarct size by approximately 35% [195]. Similar to allopurinol in a rat model of transient middle cerebral artery occlusion, its metabolite oxypurinol reduced the ischemic cerebral damage and attenuated the neurological deficits [192, 193, 196]. Allopurinol was also effective in a rabbit model of focal cerebral ischemia [197] and in hypoxic-ischemic injury models in rat pups, as well as in newborn lambs, even if its administration was delayed to the beginning of the reperfusion period [198-200]. Allopurinol, at relatively high doses, was also effective in transient middle cerebral occlusion models [190]. In a relatively mild model of stroke (20 min of ischemia and 10-90 min of reperfusion), oxypurinol produced improvements in cellular ATP levels [201]. It must also be mentioned that in some studies oxypurinol was without significant protective effects [202, 203], and the reasons for these differences between the outcome of these various studies are unclear.

Nevertheless, in vitro studies clearly demonstrate that, at least in rodents, hypoxia and reoxygenation increase the activity of xanthine oxidase and xanthine deshydrogenase, possibly due to a direct action of excitatory amino acids such as kainate [204-206]. In addition, there is some evidence that the hypoxic and reoxygenation injury to the cerebral

vasculature, especially to the endothelial cells, is dependent on xanthine oxidase [188, 189]. So far, the human therapeutic experience with allopurinol is limited to one study conducted in 22 severely asphyxiated infants [207]. In this study, allopurinol tended to improve survival, and exerted beneficial effects on free radical formation, cerebral blood flow volume, and electrical brain activity. Larger follow-up studies are required to expand on these pilot findings [208]. Interestingly, a recent study has shown increased xanthine oxidase activity being partially responsible for the generation of oxygen free radicals in a rat model of traumatic brain injury, suggesting that inhibition of xanthine oxidase may be of potential therapeutic benefit in neurotrauma [209].

Antioxidant Supplementation to Scavenge ROS

Vitamin C

Vitamin C is a potent water-soluble antioxidant in humans. Its role as a free radical scavenger and its redox relationship with other antioxidants has been the focus of continuous interest [210].

It is important to remark that differently to typical drugs, the half-life of vitamin C depends on its plasma levels. Accordingly, when plasma levels are above 70 $\mu\text{mol/L}$, ascorbate has a half-life of about 30 minutes [211]. However, in periods of deficient intake, when the renal transporters actively reabsorb the vitamin preventing acute scurvy, the plasma half-life of ascorbate is widely reported to be between 8 to 40 days [212]. Regarding absolute bioavailability, from 70 to 90 percent of usual dietary intakes of ascorbic acid (30 to 180 mg/day) are absorbed [213], although absorption decreases to about 50 percent and less with single doses above 1 gram [212, 214]. Therefore, large doses of vitamin C should be given intravenously in order to achieve high plasma concentrations of this compound.

Ascorbate uptake and content in neurons will be limited by the number of SVCT2 expressed proteins, and affinity for ascorbate becomes relevant only when ascorbate deficiency is present [215]. Indeed, vitamin C is accumulated in some cells in part by the sodium-dependent transporter with saturable kinetics [211]. Ascorbic acid is concentrated mainly in the brain and adrenal gland, among others [216, 217]. Even though glia may not normally express the SVCT2, oxidative stress due to ischemia–reperfusion injury has been shown to increase SVCT2 mRNA expression in both neurons and glia [218].

Ascorbate directly scavenges oxygen- or nitrogen-based radical species generated during normal cellular metabolism [215]. The major radical byproduct of mitochondrial oxygen metabolism is superoxide [219]. Ascorbate concentrations of 100 μM and higher are required to appreciably scavenge superoxide [220]. Vitamin C behaves as an enzyme modulator, exerting up-regulation on endothelial NO synthase (eNOS) and downregulation of NADPH oxidase [221].

Thus, vitamin C counteracts and prevents the oxidation of lipids, proteins and DNA, subsequently protecting their structure and biological function. Ascorbate has been shown to spare/recycle α -tocopherol in lipid bilayers [222] and in erythrocytes [223], therefore, enhancing vitamin E antioxidant properties. Given the lipid-rich environment of the brain, the sparing or recycling of α -tocopherol may be a very important role for ascorbate. Accordingly, when ascorbate is added to cultured cells, brain slices, and brain microsomes it prevents lipid

peroxidation induced by various oxidizing agents [224, 225], especially in combination with α -tocopherol [226, 227].

Perhaps the most dramatic acute oxidative stress in the CNS is the ischemia–reperfusion injury that occurs with ischemic stroke. Ischemia initially depletes intracellular ascorbate [228] and GSH [229] in the brain. If reperfusion with oxygen-rich blood occurs, the ROS generated by abnormal mitochondrial metabolism will extend tissue damage to areas with decreased oxidant defenses [230].

In a Chinese study, plasma vitamin C levels were inversely correlated with stroke mortality [231]. Several studies investigated the plasma vitamin C levels in patients suffering from acute stroke, finding lower levels compared to healthy controls [121, 123, 129, 232]. These studies also demonstrated that vitamin C plasma levels decrease in the first hours following cerebral ischemia [123] and tend to recover in the following days [188]. However, there are also studies that did not show any difference of vitamin C concentrations between stroke and control patients [233].

There are only three studies concerning the effect of vitamin C on the outcomes of acute ischemic stroke. In 59 stroke patients admitted to the hospital within 24 hours, Polidori and colleagues demonstrated that those randomized to aspirin 300 mg plus vitamin C 200 mg/day had slightly lower levels of lipid peroxidation markers (8,12-iPF_{2a}) compared to those taking aspirin alone [234]. In this pilot study no difference in clinical outcomes occurred between the two groups.

Ulegaddi and colleagues found that antioxidant supplementation (500 mg of vitamin C plus 800 IU of α -tocopherol) administered within 12 hours from the onset of symptoms and continued up to 14 days in acute ischemic stroke patients is able to enhance antioxidant capacity and to mitigate oxidative damage [235].

A recent study showed that vitamin C 500 mg/day IV alone resulted in increased plasma total antioxidant capacity, but it did not substantially improve the clinical and functional status of patients after 3 months [236].

Parenteral ascorbate injections decreased infarct size in both primate and rodent models of middle cerebral artery ischemia–reperfusion. When monkeys were given 1 gram/day of ascorbate parenterally for 6 days before middle cerebral artery occlusion, brain infarct size was decreased by 50% in the ascorbate-treated group compared to the control group not treated with ascorbate [237]. Ascorbate was measured in the basal ganglia of these animals and was found to increase 50% above levels in the treated compared to the untreated monkeys [237]. Thus, relatively modest increases in brain ascorbate content due to several days of parenteral administration were associated with neuroprotection against ischemia–reperfusion injury. In another primate study, high-dose ascorbate (500 mg/kg up to 2 g) was administered into the middle cerebral artery just before occlusion, which was maintained for 4 hours. At 24 hours after reperfusion, the mean infarct size in ascorbate-treated monkeys was only one third of that in untreated controls.

Pre or post-treatment with the blood–brainbarrier-impermeant vitamin C seems unable to reduce cerebral infarct volume at a dose of 500 mg/kg IV 3 hours post-middle cerebral artery occlusion [238], whereas the BBB-permeant dehydroascorbate (DHA), reduces the lesion volume by 50–90% in rats [238]. Consequently, based on the delayed rupture of BBB after cerebral ischemia, it seems important to develop molecules crossing the BBB. Recently, the selective hydroxyl radical scavenger EPC-K1, a phosphate diester of vitamins C and E able to penetrate the BBB, has been reported to reduce infarct size and lipid peroxidation, evaluated

by TBARS levels, after transient ischemia [239, 240]. Moreover, the study of Takamatsu et al. [240] indicates that the most critical period of hydroxyl radical formation for ischemic brain damage may occur a few hours after ischemia.

In a subsequent study in mice with middle cerebral artery occlusion, high-dose DHA given by intraluminal infusion injection just before, 15 minutes after, or 3 hours after occlusion markedly decreased infarct volume, mortality, and neurological deficits in mice [238]. Protection by DHA infusion was observed in both ischemia alone and ischemia–reperfusion models. A subsequent study by the same group replicated the initial results in mice and further showed that post-ischemic DHA infusion prevented the 50% decrease in brain ascorbate and also largely prevented the increase in lipid peroxidation due to ischemia–reperfusion injury [241].

Vitamin E

Vitamin E, a fat-soluble vitamin known to be one of the most potent antioxidant, acts by breaking the propagation of the free radical chain reaction in the lipids of biological membranes and reduces infarct volume by 45–55% in permanent and transient models of cerebral ischemia. Vitamin E is probably the most studied antioxidant for its potential use in the prevention and treatment of ischemic cerebrovascular disease in randomized controlled trials. The protective effects of vitamin E are more consistently found in secondary prevention trials.

A lower incidence of stroke has been demonstrated in a small number of patients with previous stroke taking aspirin (325 mg/day) and vitamin E (400 IU/day) versus those on aspirin alone [242]. The Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE study) was performed in 196 hemodialysed patients with pre-existing cardiovascular disease. In this study, participants who received 800 IU/day of natural α -tocopherol experienced a decrease of the primary combined end-points (i.e., myocardial infarction, ischemic stroke, peripheral vascular disease and unstable angina) [243].

In the largest study ever performed on the subject, the Nurses' Health Study, about 87,000 women were followed-up for an average of 8 years: participants taking vitamin E supplements had a reduced risk of ischemic stroke while they had a lower risk of coronary events [244].

Diet supplementation with vitamins, such as vitamin E, also induces a neuroprotection after ischemia [245–247]. An appreciable beneficial effect was also achieved with prophylactic oral administration of the vitamin E/lipoic acid mixture from 30 days before the ischemic event [247], suggesting that such a combination may be valuable in reducing brain damage in patients with a high risk of stroke [118]. Lipoic acid exerts its antioxidant effect by scavenging hydroxyl radicals, singlet oxygen, and nitric oxide. In addition, it chelates transient metals, recycles others antioxidants, such as vitamin E and C, and raises also intracellular levels of glutathione.

γ -tocopherol, an isoform of vitamin E, has been shown to inhibit lipid peroxidation damage and to trap reactive nitrogen species more efficiently than α -tocopherol [248]. Moreover, the administration of γ -tocopherol by bolus intravenous injection in rats immediately before and 3 hours after the MCA occlusion reduced the infarct volume more than α -tocopherol [249].

Recent studies in humans have reported an association between the plasma concentration of ascorbic acid and α -tocopherol and early clinical outcome after acute ischemic stroke [118, 129].

Supplementation with antioxidant vitamins within 12 h postinfarct after acute stroke increased plasma antioxidant capacity and reduced lipid peroxidation products. However, differences in total antioxidant capacity and MDA were only significant during the first week. This occurred in association with a predicted increase in the plasma concentration of ascorbic acid and α -tocopherol. Plasma C reactive protein was also lower in those stroke patients who received antioxidant supplements compared with controls at 7 and 14 days [250].

According to studies in stroke patients, the supply of vitamin E (727 mg/day) together with vitamin C (500 mg/day) reduced peroxidation of lipids and exerted anti-inflammatory effects [250, 251]. Hence, to define the usefulness of vitamin C for stroke therapy, its pharmacokinetics should be assessed to find the optimal daily dose, frequency and route of administration. In addition, vitamin C may work better in combination with other pro- or antioxidative agents [236].

A recent uncontrolled study of 22 ischaemic stroke patients reported an association between the plasma concentration of ascorbic acid and α -tocopherol and the degree of neurological impairment after ischaemic stroke [129]. The same study demonstrated an association between plasma total antioxidant activity and the volume of ischaemic cerebral infarction and the degree of subsequent neurological impairment.

Resveratrol

Hydroxystilbenes, naturally occurring polyphenolic compounds, are also well known for their free radical scavenging properties. Resveratrol (3,5,4V-trihydroxystilbene), one of the major components of red wine, is a representative of this group and was found to prevent lipid peroxidation [252]. Besides antioxidant activity, resveratrol may protect against brain damage via several mechanisms. Resveratrol activates the PPAR- α and PPAR- γ receptors which are recognized to have anti-inflammatory actions. This effect has been partially confirmed in PPAR- α knockout mice where resveratrol does not reduce the infarct volume after MCA occlusion [253].

To date, two studies have demonstrated that this compound could reduce the size of cerebral infarction, and this neuroprotective effect has been associated with an inhibition of rise in MDA and GSH levels induced after ischemia [254]. Recently, the naturally analogue oxyresveratrol (trans-2,3V,4,5V-tetrahydroxystilbene), which scavenges hydrogen peroxide and nitric oxide, was also shown as a potent neuroprotectant in a model of transient cerebral ischemia [255].

In mice, a single dose of resveratrol given orally 1 hour before permanent MCA ligation does not protect against ischemic damage, while given daily for 3 days before ischemia it significantly reduces the infarct size [253]. Other studies confirmed that resveratrol might reduce cerebral ischemic damage in different animal stroke models [253, 256]. Gerbils injected with resveratrol intraperitoneally experienced decreased neuronal death in the hippocampus and had lower glial cell activation either during or shortly after common carotid artery ligation and 24h later [257].

In mice, resveratrol reduces the elevated levels of metalloproteinase-9 (MMP-9), which is induced by cerebral ischemia-reperfusion [258]. By using in vitro neuronal cultures, resveratrol has been shown to induce a neuroprotective enzyme, i.e., heme oxygenase1, that is

believed to be protective by counteracting the intracellular increase of heme, a pro-oxidant agent [258, 259]. Finally, recent studies show that resveratrol may be beneficial miming ischemic preconditioning via the Silent Information Regulator T1 (SIRT1) pathway. SIRT is a NAD⁺-dependent histone deacetylase, which has been shown to inhibit stress-induced apoptosis and to increase cellular tolerance to stress [260]. During ischemic/reperfusion damage this sirtuin molecular pathway induced by resveratrol increases neuronal cell survival in an unfavourable environment [261].

N-acetylcysteine (NAC)

NAC has been reported to effectively scavenge ROS, such as hydroxyl radicals, hydrogen peroxide, and peroxy radicals, and also to increase the rate of endogenous glutathione synthesis [262]. Glutathione, which is a central component in the antioxidant defense of cells, acts either directly or indirectly as a substrate for various glutathione peroxidases to detoxify free radicals [263]. Thus, it is expected that NAC is able to reduce the volume of postischemic lesion. For instance, Carroll et al. [264] showed a 49 or 29% reduction of the lesion size, when NAC is administered prior or after the reperfusion, suggesting that the oxidative stress occurring during reperfusion exerts a more important deleterious effect than during ischemia.

A recent study demonstrated that NAC administration protects the brain from free radical injury with a therapeutic window effective up to 6 h after reperfusion, and this neuroprotection is associated with increased glutathione levels [265]. Moreover, repeated administration of NAC is more neuroprotective than a single dose, perhaps because of the short half-life of this antioxidant [262]. The administration of its monoethyl ester derivative was demonstrated to be neuroprotective for the first time after the intracerebroventricular infusion for the entire ischemia/reperfusion period [228].

Other Antioxidant Strategies

B-group vitamin supplementation immediately postinfarct may have antioxidant and anti-inflammatory effects in stroke disease independent of their homocysteine-lowering effect [251]. The important findings of this study were that supplementary antioxidants with or without B-group vitamins within a given period of time postinfarct enhanced antioxidant capacity and mitigated oxidative damage in stroke disease. No additive or synergistic effects of antioxidants and B-group vitamins together on any outcome measure were reported. All vitamin supplement groups reduced tissue inflammation [250].

COX-2 inhibitors, NS-398 and nimesulide, were also demonstrated neuroprotective after transient and permanent ischemia. Of interest in these studies is that NS-398 and nimesulide are still efficient when their administration was delayed until 24 h after ischemia [266, 267]. On the one hand, these results provide strong evidence that COX-2 may play an important role in the delayed progression of the postischemic lesion, and on the other hand, they suggest that COX-2 inhibition might represent an interesting therapeutic strategy in stroke treatment at the late phase of the damage, although it is not clear when COX-2 is induced in the progression of human cerebral infarction.

Pharmacological studies in animals showed that antioxidant molecules able to cross the blood-brain barrier, such as polyethylene glycol-conjugated superoxide dismutase and catalase [268] and lazaroids [269] reduce ischemic cerebral damage. Finally, transgenic mice overex-

pressing SOD have reduced infarct size compared with wild-type mice [270] while SOD knockout mice have an increased infarct size compared with controls [6].

CONCLUSIONS

Stroke is a major cause of morbidity and mortality among western people. Since the majority of stroke cases are non fatal, its major burden is long-term disability. ROS have been implicated in brain injury after ischemic stroke. Indeed, after vessel occlusion occurs mechanisms merge into a vicious circle accounting for increased ROS production and neuron injury. Most patients do not reach medical facilities early enough for conventional reperfusion strategies, which is the cornerstone of stroke management nowadays. Therefore, therapies targeting patients who cannot undergo thrombolysis must be considered to diminish stroke consequences.

The use of conventional therapies has yielded insufficient results in terms of outcome in stroke patients. Thus, the potential use of unconventional therapies, such as antioxidant defense system enhancement, might play a key role in the management of this disease. Indeed, considering the time course of ROS production, a combined therapy considering both inhibition of ROS-producing enzymes and increased non-enzymatic antioxidant delivery to neurons is expected to effectively diminish brain injury and improve the outcome of these patients. As it can be seen in the schematic approach to antioxidant enhancement proposed on figure 2, this sort of therapy is extensive to a larger period of time compared to reperfusion, widening the amount of eligible patients.

Antioxidant defense system enhancement constitutes a safe, low-cost and widely applicable therapy for stroke patients. Taking into account the promising beneficial effects of this therapy as well as its safeness and low-cost, it emerges as a highly cost-effective alternative for that 97% ischemic stroke patients not receiving reperfusion therapy. Further randomized, double blind, placebo-controlled clinical trials are still lacking to fully establish the usefulness of this scheme.

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Chapter 40

MECHANISMS UNDERLYING ISCHEMIC STROKE PREVENTION BY TEA (*CAMELLIA SINENSIS*)

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ABSTRACT

Some prospective cohort and case-control studies suggest that long-term consumption of tea is associated with a reduced risk of mortality from ischemic stroke. Habitual tea consumption is also associated with a reduced risk of developing hypertension (an important cardiovascular risk factor for ischemic stroke). In stroke-prone spontaneously hypertensive rats, green tea consumption attenuated increases in blood pressure which corroborated with human observational studies. Tea flavonols enhance endothelial function by increasing cerebral blood flow and protecting against oxidative stress-induced ischemia. Green tea extract and its putative flavonol, epigallocatechin gallate (EGCG), significantly reduce infarct size in surgically-induced ischemic stroke in animal models. In vivo experiments show that EGCG, a potent free radical scavenger, activates endogenous antioxidant systems which reduce oxidative stress and ameliorate the sequel of events which lead to cerebral ischemia. EGCG reduces neuronal damage caused by chemically-induced oxidative stress, thus providing protection against ischemic stroke in in vitro models. Other studies have suggested that EGCG activates Nrf2 (a transcription factor which stimulates intracellular antioxidant signalling pathways) and increases the activity of heme oxygenase-I (a potent antioxidant enzyme). These mechanisms may explain, in part, the neuroprotective role of green tea against ischemic stroke. At the molecular level, components of green tea may provide neuroprotection by regulating various receptors, including GABA_A, and modulating intracellular signalling pathways, including STAT-1. The evidence from both pre-clinical and clinical studies suggests that tea may prevent ischemic stroke.

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INTRODUCTION

For thousands of years, tea (*Camellia sinensis*) has been one of the most popular beverages in Asian countries and its habitual drinking has been attributed to the prevention of many diseases, from various types of cancer to hypertension to obesity [1]. Health benefits also include the prevention of cerebrovascular events, including ischemic stroke [2,3]. The leaves of green tea contain hundreds of natural compounds, and the putative active compounds have been identified as polyphenolic flavonoid antioxidants, known as catechins. Catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and the major flavonoid (-)-epigallocatechin-3-gallate (EGCG)[4]. These antioxidants constitute about one-third of the dry weight of green tea; and the major catechin EGCG has been extensively studied to determine its biological properties. Black tea is processed differently and the fermentation process converts catechins to more complex polyphenols, including theaflavins and thearubigins. Tea polyphenols have been touted as potent antioxidants which may explain their reputed health benefits.

Ischemic stroke occurs when there is a sudden diminution in the blood supply to the brain. Narrowing of arteries supplying cerebral tissue is accomplished by endothelial dysfunction and atherosclerotic plaque build-up which play major roles in the development of cerebrovascular ischemic events. Also, the narrowing of the carotid artery, the formation of blood clots on ruptured plaques, which may break off and form emboli, all contribute to the dramatic reduction in cerebral blood supply and precipitate ischemic stroke. It is proposed that polyphenols found in black and green tea prevent the incidence of ischemic stroke by reducing LDL-cholesterol levels, oxidative stress by direct and indirect mechanisms, LDL oxidation and key inflammatory events which lead to endothelial dysfunction, atherosclerosis initiation and propagation and plaque formation.

In this chapter the evidence regarding the preventive benefits of green and black tea in ischemic stroke is presented based on the findings of epidemiological studies, interventional studies which use standardized extracts in randomized controlled clinical studies, isolated tissue preparations, *ex vivo* models, *in vitro* cell lines and animal models of disease. The molecular mechanisms underlying the preventive effects are also presented. However, much of the research has focussed on green tea, its standardized extracts or its major catechin, EGCG.

CLINICAL EVIDENCE OF ANTIOXIDANT EFFECTS

Oxidative stress is characterized by the accumulation of reactive oxygen species (ROS) [5] which triggers intracellular events leading to protein, DNA and lipid damage [6]. It has been shown that there is a significant increase in ROS in patients with ischemic stroke [7]. Oxidation of LDL-cholesterol plays a pivotal role in the initiation and propagation of atherosclerosis, plaque development and the occurrence of subsequent cardiovascular and cerebrovascular events [8,9], such as ischemic stroke. It has been shown that habitual green tea consumption increases plasma antioxidant status and reduces free radical-induced markers of lipid peroxidation [10].

An open-labelled randomized controlled study demonstrated that the habitual drinking of green tea increased plasma antioxidant power, measured by the ferric reducing/antioxidant power (FRAP) [11]. These results were further supported by a cross-over design study including 21 healthy volunteers who were given black and green tea with or without milk as a single dose [12]. This study showed that there was substantial absorption of catechin, measured by the detection of catechin-related phenolic compounds in the urine of volunteers. In another open-labelled randomized controlled trial volunteers were given 2 cups of green tea (250mg total catechin) for 42 days. There was an increased antioxidant status in these volunteers with a significant reduction in plasma peroxides and decreased induction of DNA oxidative damage in isolated lymphocyte [13].

Another study showed that drinking 6 cups of green tea for 6 months significantly increased antioxidant status and reduced oxidative stress-related toxicities in fuel pump workers exposed to benzene [14]. In this study, green tea abolished the benzene-induced depletion of plasma glutathione and erythrocyte antioxidant enzyme levels. In yet another open-labelled controlled study, which included 14 healthy men, the consumption of green tea (6g in 600mL water daily for 7 days) increased both plasma glutathione levels and FRAP, as well as ameliorated the post-exercise increase in lipid hydroperoxidase [15].

MOLECULAR MECHANISMS UNDERLYING ANTIOXIDANT EFFECTS

Oxidative stress increases the expression of transcription factors, including redox-sensitive Nrf2, which activate intracellular signalling pathways and increase the expression of antioxidant and detoxifying enzyme systems. Nuclear translocation of Nrf2 results in its binding to anti-oxidant response element (ARE-) on the promoter regulatory sites of antioxidant/Phase II detoxifying genes. Wu and colleagues [16] showed that EGCG caused an induction of heme-oxygenase (HO-1) expression in an endothelial cell culture via the up-regulation of phosphatidylinositol 3-kinase (PI3K) and extracellular signal-regulated kinase 1/2 (ERK1/2). EGCG facilitated the nuclear translocation of Nrf2 and subsequent binding to ARE sites of antioxidant genes. This finding was further corroborated in a more recent study where EGCG was shown to induce the expression of antioxidant enzymes HO-1 and manganese superoxide dismutase (MnSOD). This was facilitated by the nuclear translocation of Nrf-2 to ARE promoter regions in human mammary epithelial cell cultures [17]. The researchers also demonstrated that Nrf-2 may possibly be phosphorylated by Akt and ERK1/2 protein kinases prior to nuclear translocation in the signal transduction pathway.

Besides HO-1 and MnSOD, EGCG regulate several other antioxidant and detoxifying enzymes via intracellular signalling pathways which are also mediated by Nrf2. In *in vivo* and *in vitro* models EGCG has been shown to induce antioxidant enzymes including glutathione s-transferase, glutathione peroxidase and catalase [18].

In the vascular endothelium, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase has been identified as a major source of reactive oxygen species. It was shown that both black and green tea polyphenols suppressed the expression of specific subunits of NADPH oxidase, while decreasing oxidative stress in bovine carotid artery endothelial cells [19]. Additionally, black and green tea polyphenols induced the expression of catalase, a

potent endogenous antioxidant enzyme. The generation of reactive oxygen species by NADPH oxidase is closely linked to the inflammatory response, and more recently, it was shown that EGCG blunted the generation of ROS by inhibiting NADPH oxidase in a mast cell line, thus preventing degranulation and release of inflammatory mediators [20].

CLINICAL EVIDENCE OF ISCHEMIC STROKE PREVENTION

A large prospective cohort study in Finland, involving 26,556 middle-aged to elderly male smokers, with an average follow-up of 13.6 years showed that the habitual drinking of more than two cups of green tea daily protected against ischemic stroke, despite the known cerebrovascular risk factor [21]. In another study, which included 6358 Japanese men and women with an average follow-up of 10 years, it was shown that habitual green tea consumption consistently lowered the risk of ischemic stroke and cerebral infarction [22].

A large matched case-control study, involving thousands of Chinese men, showed that regular tea consumption provided protection against the occurrence of ischemic stroke [23]. This was further supported by a smaller case-control study, involving 838 Chinese men, which also showed that habitual drinking of green or oolong tea was inversely associated with the incidence of stroke [24]. The authors of this study also found that besides long-term consumption, that increased daily intake provided additional protection against stroke.

A recent review of the totality of the observational epidemiological evidence, which included seven prospective cohort studies and three case-control studies, suggests that habitual tea drinking provides protection against the occurrence of ischemic stroke [25]. A meta-analysis of nine case-control studies involving 194,965 persons showed that there were significant reductions in the risk of stroke, which increased in individuals consuming three or more cups of tea daily [26].

ANIMAL MODELS OF ISCHEMIA AND MOLECULAR MECHANISMS

Theaflavin, a polyphenol isolated from black tea, decreased infarct volume in an ischemic mouse model. In this model the middle cerebral artery was occluded for two hours and reperfusion over the next 24 hours and theaflavin was shown to modulate the ischemia-induced inflammatory process [27]. It was demonstrated that signal transducer and activator of transcription-1 (STAT-1), a signal transducer and activator of transcription proteins linked to various inflammation-related genes, was down-regulated by theaflavin. Theaflavin-induced inhibition of STAT-1 caused a down-regulation of inflammation-related adhesion molecules (ICAM-1) and reduced the activity of pro-oxidant enzymes (COX-2 and iNOS). These molecular events would lead to reduced inflammation and oxidative stress in the acute ischemia and could explain the reduced cerebral infarct volume in the animal model.

It was also demonstrated that theanine, a green tea polyphenol, reduced cerebral infarct size and altered the expression of biochemical markers of ischemia/reperfusion. It was shown that theanine modulated several molecular pathways which utilize gamma-aminobutyric acid_A (GABA_A) receptors and altered the expression of several genes to provide neuroprotection in this animal model [28]. A more recent study in an experimental model of ischemic stroke in

the mouse showed that the green tea extract, epicatechin, significantly reduced both the extent of cerebral infarction and neurological damage when given before or shortly after reperfusion [29]. Epicatechin caused an up-regulation of transcription factor Nrf2 and increased activity in the antioxidant enzyme heme oxygenase-1 (HO-1) activity and these events may be important in providing protection against ischemic damage.

A cascade of inflammatory events occurs at the onset of ischemic stroke, such as the increased expression of intracellular adhesion molecule-1 (ICAM-1), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), which promotes macrophage recruitment, production of inflammatory mediators and oxidant-triggered endothelial dysfunction. Theaflavin decreased infarct volume by suppressing the expression of ICAM-1, iNOS and COX-2, by the inhibition of STAT-1. Similarly, ECGC was used in a rat model which showed that there was an inhibition of the ischemic/reperfusion-induced activation of STAT-1 [30]. It was also shown that ECGC inactivated STAT-1 by specifically binding to the phosphorylation site on STAT-1, blocking down-stream signalling pathways responsible for the transcriptional induction of pro-inflammatory mediators.

CLINICAL EVIDENCE OF ANTIHYPERTENSIVE EFFECTS

Hypertension is a major risk factor for the development of ischemic stroke. Hypertension increases the likelihood of endothelial dysfunction and accelerates the events leading to atherosclerotic initiation and progression. The habitual drinking of green tea has been proposed to reduce the risk of hypertension and hypercholesterolemia which are major risk factors associated with the development of ischemic stroke.

A meta-analysis of five randomized-controlled trials including 343 volunteers (some of which were hypertensive) showed that drinking green tea regularly for at least four 4 weeks produced a statistically significant reduction in blood pressure [31]. A larger meta-analysis including 9 studies, which included 4,378 stroke incidents among 194,965 individuals, showed that drinking more than 3 cups of green tea on a daily basis significantly reduced the risk of ischemic stroke [32].

An observational study in 1507 newly diagnosed hypertensive patients (without a history of hypertension over the last 10 years) showed a protective effect in habitual green tea drinkers [33]. A large observational study including 40,530 persons in Japan followed for up to 11 years also demonstrated a significant risk reduction in cardiovascular disease, including stroke, with habitual green tea consumption [34,35]. More recently, a 5-year follow-up observational study in 6,358 individuals demonstrated that green tea consumption significantly reduced the risk of stroke in a dose-dependent manner [22].

A randomized controlled trial involving 110 healthy subjects given a standardized green tea extract twice daily demonstrated significant reductions in cardiovascular risk factors, including blood pressure, LDL cholesterol and oxidative stress over a 3-month period [36]. A small open-labeled randomized crossover study in 20 healthy male smokers showed that the consumption of 400mL of green tea induced immediate reversal of endothelial dysfunction as measured by forearm blood flow [37].

CLINICAL EVIDENCE OF ANTI-HYPERCHOLESTEROLEMIA EFFECTS

An open-labelled randomized controlled study lasting 42 days showed that the consumption of two cups of green tea (250mg catechins) caused a modest, but significant, reduction in LDL cholesterol [13]. Another study was conducted over 24 weeks and had a stratified randomized placebo-controlled trial design. In this study, 40 overweight Japanese children who consumed either green tea (containing 576mg catechin) or placebo and it was demonstrated that green tea produced a significant reduction in LDL cholesterol [38]. Another randomized-controlled trial with 111 participants given either a standardized green tea extract or placebo showed that green tea produced a significant decrease in both total and LDL cholesterol in volunteers with initial elevated LDL cholesterol [36]. A meta-analysis of four interventional studies showed that green tea flavonoids caused a reduction in LDL cholesterol [39].

A randomized controlled study in 40 healthy volunteers showed that the daily intake of 400mg catechin (equivalent to 6-7 cups of green tea) caused a significant reduction in oxidized LDL, a major player in the initiation of atherosclerosis, although there were no effects on the overall lipid profile [40]. Subsequently, a small open-labeled crossover study in 5 healthy females showed that the consumption of 4.5g daily of ground green tea over 2 weeks significantly reduced the susceptibility of LDL oxidation *ex vivo* [41].

In a small study including 12 healthy volunteers the daily consumption of 600mL of green tea over four weeks had no effect on lipid profile, total antioxidant capacity or on markers of inflammation [42]. However, the levels of oxidized LDL and soluble vascular cell adhesion molecule-1 (sVCAM-1) were significantly reduced.

A study using cultured human liver (HepG2) cells showed that EGCG lowered LDL levels by promoting an increase in LDL receptor density and binding activity by modulating gene expression [43]. The green tea polyphenol also induced sterol regulatory binding protein-1 (SREBP-1) activity, which modulates intracellular metabolism of cholesterol.

ANIMAL MODELS OF HYPERTENSION AND MOLECULAR MECHANISMS

Black and green tea intake over three weeks attenuated blood pressure increase in stroke-prone spontaneously hypertensive rats, by reducing oxidative stress and levels of plasma nitric oxide [44]. Furthermore, green tea produced an increase in protein expression of the antioxidant enzyme catalase. It was shown that both black and green tea caused an inactivation of myosin light chain, which plays a pivotal role in vascular smooth muscle contraction and increase vascular resistance, and this may be key in the attenuation of blood pressure rise. The consumption of green tea extract from an early age in malignant stroke-prone spontaneously hypertensive rats significantly reduced the rate of blood pressure increase over time and delayed the onset of spontaneous stroke [45].

Angiotensin II (Ang-II), a potent endogenous vasoconstrictor, causes oxidative stress at high levels and it is implicated in endothelial dysfunction associated with the development of hypertension and atherosclerosis. Rats given green tea extract in their drinking water and injected with Ang-II over 13 days were less likely to develop hypertension [46]. It was shown

earlier that EGCG inhibited Ang-II-induced vasoconstriction by inhibition of the c-Jun N-terminal kinase (JNK) signalling pathway involved in the transcriptional regulation and post-translation modification of mediators which regulate vascular tone [47].

MECHANISMS UNDERLYING EFFECTS IN THE INITIATION AND PROPAGATION OF ATHEROSCLEROSIS

The initiation and propagation of atherosclerosis is a complex inflammatory process commencing with vascular injury, exposure of endothelium accompanied by adhesion and migration of monocytes into the intima. Oxidized LDL-cholesterol play a major role as macrophages are transformed into foam cells. The concerted interactions between transcription factors, intracellular signalling pathways and numerous pro-inflammatory cytokines, adhesion molecules and chemotactic molecules are vital to the formation of atherosclerotic plaques. These events further promote atherosclerotic progression resulting in endothelial dysfunction and plaque formation [48]. Theoretically, antioxidant polyphenolic compounds found in black and green tea should reduce LDL cholesterol oxidation and attenuate atherosclerotic plaque formation to thwart long-term cerebrovascular events, such as ischemic stroke.

The lecithin-like oxidized low-density lipoprotein receptor-1 (LOX-1) is the primary receptor for oxidized LDL in the vascular endothelium, and its activation induces intracellular signals leading to the cascade of pro-inflammatory events. Recently, it was shown that EGCG significantly suppressed the expression of LOX-1 receptors at the transcriptional level in human umbilical vein endothelial cells. This causes the subsequent suppression of intracellular signals, mediated by the redox-sensitive transcription factor NF- κ -B, which up-regulates the expression of pro-inflammatory genes and cell adhesion molecules [49]. It was also shown that protein kinases, p38 MAPK and Ark, responsible for the activation of NF- κ -B were inhibited in the presence of EGCG.

Monocyte chemotactic protein-1 (MCP-1) plays an important role in the migration, adhesion and infiltration of monocytes to the site of vascular injury in the initiation and propagation of atherosclerosis. This cytokine also plays a key role in medial thickening at the site of atherosclerotic lesions. In a randomized clinical trial, involving 30 otherwise heavy smokers, high dose green tea (580 mg catechins daily) over two weeks produced a significant decrease in MCP-1 [50].

Pre-treatment with EGCG inhibited phorbol 12-myristate 13-acetate (PMA)-induced MCP-1 expression and transcription in endothelial ECV304 cells. It was also shown that EGCG inhibited the expression of transcription factors NF- κ B and AP-1, which regulate the expression of MCP-1 and several pro-inflammatory cytokines [51]. An *in vitro* study using a human umbilical vein endothelial cell line showed that EGCG inhibited the expression of vascular cell adhesion molecules (VCAM-1) induced by the pro-inflammatory cytokines TNF- α and IL-1 β [52]. Recently, another study using the human monocyte cell line THP-1 showed that EGCG suppressed the gene expression of both monocyte chemotactic protein-1 (MCP-1) and monocyte cell surface receptors which facilitate migration of monocytes in the arterial wall [53]. It was also shown that EGCG inhibited the cell surface expression of activated β 1 integrin adhesion molecules.

Tumour necrosis factor- α (TNF- α) is an important pro-inflammatory cytokine with a central role in the complex processes in atherosclerotic plaque formation, and EGCG was shown to inhibit TNF- α -induced production of MCP-1 in an endothelial cell culture [54]. It was also demonstrated that EGCG inhibited the phosphorylation of the downstream protein kinase Akt in the intracellular signalling pathway.

Oxidized LDL-cholesterol trapped in vascular endothelium triggers a cascade of inflammatory processes mediated by the pro-inflammatory transcription factor NF- κ B. A recent study showed that green tea polyphenols blocked the oxidized LDL-induced up-regulation of NF- κ B and inhibited the pro-inflammatory cytokine TNF- α production in human umbilical vein endothelial cells [55].

EGCG decreased the vascular endothelial expression of fractalkine, an adhesion molecule and chemoattractant involved in the initial stages of atherosclerosis, in human umbilical vein endothelial cells stimulated with TNF- α [56]. It was shown that down-regulation of fractalkine was mediated by suppression of NF- κ B.

EFFECTS OF TEA POLYPHENOLS ON INFLAMMATORY CYTOKINE PRODUCTION IN ATHEROGENESIS

Besides being a lipid disorder, it is now well established that atherosclerosis is a chronic inflammatory disease. Several pro-inflammatory mediators, known as cytokines, are pivotal in the initiation and maintenance of the inflammatory atherosclerotic state [57]. The pro-inflammatory cytokine interleukin-12 (IL-12) is involved in the early response to stimuli and forms a bridge between innate and adaptive immunity and plays a key role in atherogenesis. It was demonstrated that green tea catechins, particularly EGCG, inhibited the bacterial lipopolysaccharide-induced IL-12 production in murine peritoneal macrophages and a macrophage cell line [58]. This was facilitated by suppression of upstream intracellular pathways, including inhibition of p38 MAPK, which activate and promote the subsequent DNA binding of NF- κ B to promoter regions of other pro-inflammatory cytokines.

Tumor necrosis factor- α (TNF- α) is a potent pro-inflammatory cytokine which plays a pivotal role in augmenting the inflammatory response via the intracellular transcription factor NF- κ B. It was demonstrated the EGCG inhibited lipopolysaccharide-induced TNF- α gene expression and protein production in macrophage cell cultures by blocking the activation of the pro-inflammatory transcription factor NF- κ B [59]. It was further elucidated that EGCG inhibited NF- κ B nuclear translocation, and subsequently increased the expression of pro-inflammatory proteins, by suppressing upstream signalling events involving the cytosolic inhibitory protein I κ B[60]. Also, EGCG was shown to inhibit phorbol 12-myristate 13-acetate (PMA)-induced production of pro-inflammatory cytokines TNF- α , IL-6 and IL-8 in a human mast cell line [61]. EGCG prevented the activation of NF- κ B and ERK1/2 in pro-inflammatory intracellular signalling pathway.

In monocytes, isolated from human blood, it was shown that green and black tea extracts inhibited the lipopolysaccharide-induced expression of the pro-inflammatory cytokines IL-1 β , IL-6, IL-8 and TNF- α and prostaglandin E₂ [62]. These researchers also showed using a macrophage cell line that both tea extracts inactivated NF- κ B via inhibition of proteasome function. Another study showed that there was a transcriptional induction of IL-6 in a

macrophage cell line incubated in the sera of normocholestraemic hypertensive individuals [63]. This study provided the evidence that hypertension induces mechanisms which attenuates inflammatory processes linked to the initiation of atherosclerosis.

EFFECTS OF TEA POLYPHENOLS ON VASCULAR ENDOTHELIUM FUNCTION

Persistent endothelial dysfunction, caused by oxidative stress, is crucial in the pathogenesis of atherosclerotic plaques which eventually progress to events such as ischemic stroke and myocardial infarction. It has been proposed that polyphenols in tea affect vascular endothelium function by improving blood flow which increases and maintains cerebral perfusion which would prevent ischemic stroke [64].

In a randomized cross-over trial it was demonstrated that green tea caused an acute increase of almost 4% in flow-mediated dilatation of the brachial artery [65]. Smoking is the most important risk factor for the development of atherosclerosis and a recent randomized controlled study showed that daily consumption of high dose green tea catechins (580 mg) for two weeks in heavy smokers produced a significant improvement in acetylcholine-mediated endothelial responsiveness in the forearm following acute and chronic dosing [50]. The investigators also demonstrated that green tea at the high dose increased levels of the endogenous endothelium-derived vasodilator nitric oxide.

MECHANISMS UNDERLYING THE EFFECTS OF TEA ON ENDOTHELIAL FUNCTION

In spontaneously hypertensive rats EGCG given over a three-week period attenuated the rise in systolic blood pressure by improving endothelial dysfunction [66]. Earlier it was shown that EGCG produced an endothelium-dependent vasodilation caused by an increase in endothelial-derived nitric oxide synthase (eNOS) activity via activation of Akt and protein kinase A (PKA) in bovine aortic endothelial cells [67]. Similarly, EGCG was shown to induce eNOS in *ex vivo* mesenteric vascular beds and bovine endothelial cells [68]. These experiments indicate that EGCG in the presence of reactive oxygen species and Fyn utilized intracellular signalling pathways which led to the activation of phosphatidylinositol 3-kinase (PI-3), Akt and eNOS. Extracts from both black and green tea stimulated eNOS activity in bovine aortic endothelial cells and produced significant relaxation of isolated rat aortic rings [69]. Green tea catechins decreased endothelial exocytosis, the initial inflammatory step in atherogenesis, by attenuating monocyte adhesion to vascular endothelial and increased nitric oxide by enhancing eNOS activity [70].

Caveolin-1 negatively regulates the expression of eNOS, and it was shown that green tea polyphenols down-regulated the expression of this regulator in bovine aortic endothelial cells [71]. This down-regulation, which caused an increase in eNOS expression, was facilitated by the activation of ERK 1/2 and inhibition of p38MAPK signalling pathways.

On the other hand, related to atherosclerosis, the expression of inducible nitric oxide synthase (iNOS) promotes the production of inappropriate levels of nitric oxide which

initiates a vascular immune response to activated macrophages. In another study, EGCG blocked the angiotensin-II-induced expression of iNOS in human umbilical vein endothelial cells [72]. An earlier study using cancer cell lines showed that EGCG down-regulated the both the expression of iNOS mRNA and protein, as well as nitric oxide production, by suppressing the activation of STAT-1 α transcription factor [73].

CONCLUSIONS

Tea has been one of the most popular beverages for millennia and many health benefits have been attributed to its antioxidant effects. Many observational and interventional studies have shown that regular tea consumption over the long term has beneficial effects in lowering cardiovascular risk factors, such as blood pressure and LDL-cholesterol levels, and improve endothelial function. These are important biochemical and physiological factors which contribute to atherosclerotic development and the occurrence of cerebral ischemia.

Catechins and theaflavins are important antioxidant polyphenolic compounds identified in green and black tea respectively. Although these antioxidants act directly to scavenge reactive oxygen species they also trigger other molecular mechanisms which enhance their antioxidant activity. Tea polyphenols activate intracellular signalling pathways utilizing the redox-sensitive Nrf2 transcription factor and a complex network of protein kinases. This interaction up-regulates the expression of potent endogenous antioxidant enzymes, including catalase and heme oxygenase-1, and blunts other signalling pathways which enhance the production of reactive oxygen species.

Tea polyphenols reduce the oxidation of LDL-cholesterol, which is a key component in the development of atherosclerotic plaque formation, and blunt inflammatory processes necessary for initiation and propagation of atherosclerosis. In *in vitro* studies it has been demonstrated that tea polyphenols attenuated many processes necessary for monocyte attraction, adhesion and migration at the site of injury on the vascular endothelial. In experimental models catechins suppress the release of chemoattractants, pro-inflammatory cytokines and adhesion molecules by complex intracellular mechanisms which utilize the pro-inflammatory NF- κ B transcription factor.

Catechins have also been shown to improve endothelial function by causing vascular relaxation. This activity would be beneficial to increase blood flow and reduce the likelihood of ischemia. Experimental models show that catechins cause vasodilatation by increasing endothelial nitric oxide concentrations via induction of endothelial-derived nitric oxide synthase (eNOS). Tea catechins activate intracellular signalling pathways which utilize a complex series of protein kinases which up-regulate the expression of eNOS.

Although most *in vitro* and *in vivo* studies indicate biological activity and the modulation of various intracellular signalling pathways, it must be borne in mind that most of these experiments use tea polyphenol concentrations much higher than that which could be achieved by moderate tea consumption.

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Chapter 41

NEONATAL ASPHYXIA AND STROKE: MORBIDITY, MODELS, CONSEQUENCES, AND TREATMENTS

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ABSTRACT

Neonatal and early childhood asphyxia is a major cause of morbidity and chronic cognitive dysfunction. In developed countries approximately births per 1000 have asphyxia related complications including encephalopathy.

In this chapter we will discuss the burden of neonatal asphyxia and stroke, and the neurological consequences to the individuals afflicted, including epilepsy, cerebral palsy, and cognitive impairment.

At present, treatment is focused on managing the symptoms of perinatal asphyxia. As new treatments are clearly needed, various models have been developed to test putative neuroprotectants.

We discuss the strength and limitations of each of the most commonly used animal models.

We finish with a discussion existing treatments for acute care, including hypothermia, and the most promising pharmacological candidates for clinical use.

1. INTRODUCTION

1.1. Peri/Neo-Natal Hypoxia and Hypoxia Ischemia

Hypoxia is pathologically low blood oxygen levels (Bracci, Perrone, and Buonocore, 2006) and ischemia is disruption in blood flow. Ischemia due to the occlusion of a blood vessel in the brain results in a stroke; a cascade of neuronal death that is extremely common in aging adults (del Zoppo, 1998). Given that the damage caused by ischemia is the result of hypoxia, why are the two terms used to describe some types of ischemia but not others? Why

is the designation “hypoxia ischemia” most often associated with neurological damage in newborns, either around the time of birth (perinatal) or recently after birth (neonatal)? The answer is in the history of research into neonatal hypoxia, a condition in which ischemia causes not only local hypoxia but also global hypoxia throughout the fetus. The fetus relies on nutrients and oxygen supplied through blood vessels of the umbilical cord, which can become compressed during birthing complications and cause ischemia of the umbilical cord, resulting in global hypoxia of the fetus and damage to sensitive organs (Satas, et al., 2003). The brain is injured significantly more than other organs and these effects are lasting with significant effects of quality of life (Gunn, Gunn, deHaan, Williams, and Gluckman, 1997; Hopkins-Golightly, Raz, and Sander, 2003). The pathology is very similar to that caused by local ischemia in the brain (stroke) (Comi, Weisz, Highet, Johnston, and Wilson, 2004). This unique combination of ischemia and global hypoxia gave rise to the field of hypoxic ischemia in the peri/neo-natal period of life. This chapter will discuss the properties of peri/neo-natal hypoxia ischemia with respect to neuro-pathology, the animal models used to investigate this pathology, and potential interventions and pharmacological treatments that are under development.

1.1.1. Hypoxia Ischemia Prevalence and Risk Factors

Peri/neo-natal hypoxia is a pathological drop in oxygen levels in an infant’s blood, often caused by compression of the umbilical cord or asphyxia due to birthing complications or a choking incident (Lawn, Shibuya, and Stein, 2005). In 1997 it was estimated by the World Health Organisation (WHO) that 19% of the 5 million infant deaths that year were caused by hypoxia related condition (Shiffman, 2010). The most prevalent birthing complication that results in hypoxia ischemia is nuchal cord; this involves the umbilical cord forming a loop around the neck of the fetus, and has been linked to an increase in perinatal mortality. During nuchal cord the umbilical cord can become compressed, blocking the supply of oxygenated blood to the fetus. This can cause acidosis of the fetal blood and hypercarbia (Hankins, Snyder, Hauth, Gilstrap, and Hammond, 1987). Nuchal cord occurs in 8-34.5% of births, with around 5% of all births having a “tight” nuchal cord (Ogueh, et al., 2006). Due to advances in intrapartum care most of these cases are asymptomatic. A study by Ogueh et al. (2006) analyzed data from 57,873 births and found that tight nuchal cord incidents resulted in lower birth weights, longer birthing times, increased incidences of shoulder dystonia, and increased cesarean intervention. These are often associated with ischemia and encephalopathy (Bloch, 2005).

In the developed world the incidents of such complications have decreased dramatically over the last three decades. In Canada between 1991 and 2004 the rate of hypoxia ischemia related complications dropped from 43.8 to 2.4 cases in every 1000 births (Dzakpasu, et al., 2009). This decrease is due to improved care with a rise in the use of fetal heart monitoring systems (cardiotocography) and induced labor and caesarian births (Smith, Wells, and Dodd, 2000). In the developing world such data is difficult to acquire as stillbirths (including hypoxia related deaths) are often not reported and no birth or death certificate is generated. However it is likely that the rate of asphyxia related complications is much higher than that of developed countries (Lawn, et al., 2005).

Stroke is a disruption in the blood flow within the brain. This can be caused by a thrombus (ischemic stroke) or a rupture of a cerebral blood vessel (hemorrhagic stroke). Stroke during the perinatal and neonatal period was previously thought to be a relatively rare

pathology, but recent evidence has suggested a far greater prevalence. Peri/neo-natal stroke has gone unnoticed due to plasticity of the neonatal brain. Because of the recent emergence of this field and because of the physiological similarity to the hypoxia ischemia, peri/neo-natal stroke has come under the research umbrella of hypoxia ischemia, sharing many animal models and putative therapies. Lynch et al. (2002) reviewed perinatal stroke which they defined as the developmental period between 28 weeks gestation to 28 days postnatal age and calculated the incidence of symptomatic perinatal stroke to be 1 in every 4000 births in the developed world. However, the authors report that because of the difficulty of diagnosis due to difficulty in assessing newborns for neurological function, the incidence of symptomatic stroke may be higher still. Because stroke can occur in any region of the brain the symptoms are more varied than that of hypoxia ischemia, and include motor deficits, sensory deficits, impairment of memory and cognition, and epilepsy (Kolk, Ennok, Laugesaar, Kaldoja, and Talvik, 2010).

2. CELLULAR PROCESSES OF NEURONAL DEATH DURING ISCHEMIA

2.1. Excitotoxicity

The reduction in oxygen levels and glucose levels in the brain following HI causes disruption in neuronal ATP production. This causes the mechanisms which maintain the cell membrane potential such as Na^+/K^+ ATPase to cease functioning, causing the cell to depolarize and release its neurotransmitters including glutamate (reviewed in (Nishizawa, 2001; J. R. Rivers and Ashton, 2010a; Rothman and Olney, 1987). Increasing concentrations of glutamate in the extracellular space causes excitation of other neurons, primarily through NMDA and AMPA receptors. This not only increases the energy demands of cells in an already energy deprived environment, but also results in pathological levels of Ca^{2+} flux into the neurons. This causes osmotic influx of water resulting in cellular edema that can result in necrosis as well as disruption in mitochondrial function, resulting in apoptosis (Wahlgren and Ahmed, 2004). As the neurons depolarize and undergo necrosis, further glutamate is released resulting in an excitotoxic cascade throughout the brain. This process occurs in both the immature and mature brain. However, recent evidence suggests that NMDA receptors in the developing brain have enhanced function, and that this is crucial for plasticity during development. This increases susceptibility to excitotoxicity in the immature brain (Johnston, et al., 2009). The ability of astrocytes to absorb high levels of neurotransmitters is reduced in the immature brain, with cerebral spinal fluid (CSF) levels of glutamate shown to be heightened in HI affected infants up to 16 hours after the insult (Pu, et al., 2000).

2.2. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

ROS and RNS are highly reactive molecules that can react with cellular components including proteins, fats, and nucleic acids. In the case of proteins this causes a breakdown of cellular process including enzyme function, ion pump function and receptor activity, and can

result in depolarization of the cell, potentially initiating the excitotoxic cascade. In the case of fats such as phospholipids, membranes can become permeable, also resulting in depolarization of the cell and of the mitochondria, causing a loss of energy production. This reaction is especially damaging as the phospholipids auto-catalyze the oxidation of other phospholipids resulting in widespread membrane failure. This can lead to further cellular edema and excitotoxicity. Oxidative damage to nucleic acids can result in mutation and a loss in translational and transcriptional function (Ikonomidou and Kaindl, 2011).

ROS and RNS are continuously produced by cells but are normally in balance with endogenous antioxidant mechanisms that safely catalyze the reactions of ROS and RNS to non-harmful compounds. For example, the highly reactive ROS superoxide reacts with water to form hydrogen peroxide, catalyzed by superoxide dismutase. Hydrogen peroxide is then reduced with oxygen to form water, catalyzed by catalase (Ikonomidou and Kaindl, 2011). A major site of ROS and RNS production is mitochondria. Electrons in the electron transport chain (ETC) normally contribute to a controlled series of reactions, but can become dissociated from the ETC before reaching the critical molecule complex IV, and instead react with other nearby molecules. These uncontrolled reactions form ROS and RNS; for example electron donation to oxygen produces superoxide.

If the balance between ROS, RNS and endogenous antioxidant mechanisms is disrupted, ROS and RNS can react with mitochondrial membrane phospholipids and disrupt the integrity of the membrane. This causes depolarization and a catastrophic reduction in of ATP synthesis. This also causes cytochrome C to leak from the membrane into the cytosol, and act as signaling molecule for apoptosis. During HI, ROS and RNS levels rise beyond antioxidant capacity, and through this pathway cause neuronal apoptosis for days after injury.

In HI, low oxygen levels prevent the donation of the electrons of the ETC, this causes electrons to build up on the ETC and leak from the chain, generating ROS and RNS. Once HI is over, reperfusion produces high levels of oxygen which results in uncontrolled electron donation to oxygen, generating more superoxide and other ROS and RNS. Until recently 100% oxygen was given to infants that had undergone an HI incident. It is now recognized that 100% oxygen may exacerbate reperfusion injury and that normal levels of oxygen may be less harmful (Pu, et al., 2000).

The developing brain is more sensitive to increases in ROS and RNS than the mature brain. One reason for this is because the developing brain has lower levels of antioxidants. One study has show that fetal serum levels of the strong antioxidant tocopherol were 80% lower than that of an adult. Similar deficiencies have been found for other antioxidants such as selenium. The developing brain is also restricted to the extent that it can upregulate antioxidant synthesis or function; for example, glutathione peroxidase and glutathione. Contradicting this however, some evidence suggests that in healthy full term births the ratio of antioxidant to ROS/RNS is greater than that of an adult, perhaps because of adaptive mechanisms that help the newborn adapt to the high oxygen environment outside the womb (Parmigiani, Gianotti, Pezzoni, Corradi, and Bevilacqua, 2011). However, this may not be true for premature infants, which is particularly significant because of the common co-morbidity of premature birth with perinatal HI (Drury, Nycyk, Baines, and Cooke, 1998).

2.3. Inflammation

Under healthy conditions the brain is an immune privileged organ. Specialized glia cells called microglia, which act as the resident immune cell for the CNS, become activated upon the molecular recognition of tissue damage or the presence of pathogens. Once activated microglia secrete proinflammatory cytokines which promote the migration and activation of more microglia and cause the endothelium to express adhesion molecules that allow blood-borne immune cells to infiltrate into the CNS. Once at the site of injury, immune cells produce a cocktail of neurotoxic factors that includes ROS/RN, cytokines, and glutamate.

These diffuse from the immediate site of injury (infarct core) into the surrounding tissues, generating further neuronal death in the proximal healthy tissue (penumbra). Immune cells produce NO from the oxidation of L-arginine to citrulline via various nitric oxide synthase (NOS) enzymes. NO has both vasodilatory and antimicrobial effects, but when produced at pathological levels can cause neuronal damage by the activation of apoptosis pathways and competition for oxygen with cytochrome c (Klegeris, Bissonnette, and McGeer, 2003).

The cytokines produced by microglia and systemic immune cells have been shown to be neurotoxic in many *in vitro* studies (Klegeris, Bissonnette, and McGeer, 2003; Venters, et al., 1999). Cytokines including TNF α and IL1 β induce astrocytes to produce NO via inducible NOS (iNOS) and affect cell signaling pathways involved in neuronal survival and apoptosis (J. K. Lee, Tran, and Tansey, 2009; Venters, et al., 1999). Infections during pregnancy can produce HI-like processes, and evidence suggests that cytokines produced by the mother can have neurotoxic effects on the fetus (Bloch, 2005).

NO produced by activated immune cells and astrocytes induces both the release of glutamate by neurons via depolarization and the inhibition of the reuptake of glutamate by astrocytes (Bal-Price and Brown, 2001; Chao, Hu, Ehrlich, and Peterson, 1995).

Clear evidence of the role of neuroinflammation in neuronal death after has been provided by the use of transgenic mice that lack key proinflammatory cytokines, interleukin 1 β and interleukin 18. The transgenic mice were protected from hypoxic injury compared with wild type controls (Felderhoff-Mueser, et al., 2005).

However, clinical studies of neuroinflammatory diseases, such as meningitis, demonstrate that neuroinflammation can occur without neuronal death, so oxidative injury may be necessary to initiate inflammation that results in neuronal death.

3. BRAIN REGIONS VULNERABLE TO THE EFFECTS OF HYPOXIA ISCHEMIA

3.1. Prenatal and Perinatal Hypoxia Ischemia

The regions and cell types that are most vulnerable to damage change with the development stage of the brain. Prenatal and perinatal HI is associated with white matter injury such as periventricular leukomalacia (PVL) and periventricular white matter injury (PWMI) (Scafidi and Gallo, 2008). The prevalence of PVL and PWMI decreases with increased birth weight and length of pregnancy, with a preterm prevalence of 60 PVL infants per 1000 births compared to a full term prevalence of 1.3/1000 (Bracci, et al., 2006). The

particular stage of cell development and receptor types expressed determines sensitivity to injury.

In prenatal and perinatal HI the insult occurs during production of oligodendrocytes when oligodendrocytes progenitor cells (OPC's) are immature and highly active (Bracci, et al., 2006). During this stage, OPC's express an intermediate form of the AMPA receptor that lacks the GluR2 subunit.

This facilitates higher levels of Ca^{2+} transport, and makes the developing OPC's more susceptible to excitotoxicity (Johnston, et al., 2009). There is also evidence that the OPC's have low levels of antioxidant enzymes (Ikonomidou and Kaindl, 2011).

3.2. Neonatal Hypoxia Ischemia

In a full term infant of normal birth weight, oligodendrocytes production is advanced, but myelination of neurons is still at an early stage. Hypoxia during this period has little effect on the white matter, but does have substantial effects on neurons in the basal ganglia (Rice and Barone, 2000). Neonatal HI causes damage predominately to the putamen and the thalamus, and this can result in cerebral palsy; general non-progressive brain damage that occurs during development and predominately affects motor function. MRI studies suggest that the susceptibility of motor centers is due to high levels of glutamatergic input (Johnston, Trescher, Ishida, and Nakajima, 2001). This is further supported by fMRI studies that show newborns that have HI induced seizures have hyperactive neurons in these regions (Johnston, et al., 2001).

The hippocampi are key structures involved in spatial memory, and are vulnerable during neonatal hypoxia (Bass, et al., 2004). Although studies in humans are limited, it appears that hippocampi are among the first regions to be affected during HI. MRI and functional studies show that children that have undergone HI during or near birth have near normal intelligence with only mild dyspraxia can have severely affected memory and hippocampal volume (Gadian, et al., 2000). Little is known about the processes that cause hippocampal sensitivity despite extensively studies using rodent models of HI, although there is some suggestion that the hippocampus may be a site of intense excitotoxicity (Clarkson, et al., 2005; Gurd, et al., 2002). Complicating matters, these regions may be especially plastic during early development, potentially masking damage from behavioural assessment (Johnston, et al., 2009).

Interestingly, mechanisms of cell death change with development, with a shift from programmed cell death early in development to necrosis in the mature brain (Liu, Siesjo, and Hu, 2004). This suggests that targeting apoptosis for pharmacological intervention may be more successful in the developing brain than in the mature brain (Cowper-Smith, Anger, Magal, Norman, and Robertson, 2008).

4. ANIMAL MODELS OF HYPOXIA ISCHEMIA

Many animal models have been used to study peri/neo-natal HI. The most common animal models use sheep, pig or rodents. A review in 1996 found that 26% of all animal

studies on HI were done using rodent models, with sheep and pig models representing 23% and 22% of the research respectively (Roohey, Raju, and Moustogiannis, 1997). Critically, this review found that 71% of all studies assessed the outcomes of the HI only between 0 and 24 hours after the insult. Recent evidence suggests that disease progression is variable and can last for weeks or months after the initial insult (Kolk, et al., 2010; Vannucci and Vannucci, 2005). This has historically been a shortcoming of animals studies and has only recently started to be addressed (Vannucci and Vannucci, 2005). This section will give a brief description of some of the common animal models, addressing the advantages and disadvantages of each.

4.1. Primate

Primate models have the advantage of close homology to humans. The genetic profiles of the animals are similar to humans as well as the gestational process and development. The complexity of the brain is the most closely related to humans out of any animal model (Roohey, et al., 1997). Primate models normally involve inducing injuries either prior to birth or at birth. This is because brain development is very similar to the human condition so unlike other animal models such as rodent models, the estimated equivalent developmental stage of the fetus at birth is the similar to humans. Two models dominate the primate field; the most common involves cesarean birth where the umbilical cord is occluded for 10 to 15 minutes (Beckstrom, Humston, Snyder, Synovec, and Juul; Juul, et al., 2007). The second model involves warm saline emersion after birth prior to the first breath of the infant (Roohey, et al., 1997). Both models result in very similar histological and behavioral pathological effects to that of the human condition, such that putative therapeutics that have been found to be effective in these models should be strongly considered for human trial.

The limitation of primate models involves expense and accessibility. The cost of primate models and the long gestational periods of the animals constrains experimental numbers, thus the studies are often underpowered. This is a particular problem in animal models of hypoxia, where highly variable outcomes are the norm (Jack R. Rivers, Sutherland, and Ashton, 2011). Ethical issues arise for the same reason that makes non-human primates excellent models of human pathologies. It is likely that these animals have a greater similarity to humans in experiencing suffering and pain than other animals. For this reason experiments on primates are often the target political and social debate, limiting their use in some countries (McNeil, 2008).

4.2. Sheep and Pig

Pigs and sheep are also used in models of peri/neo-natal HI. Both pigs and sheep have similar sized newborns to humans, with comparable stages of brain development and size. These factors result in the oxygen and other metabolic demands of the brain that are similar to humans (Northington, 2006). There are several different methods of inducing HI in these animals. Hypoxia of the pregnant mother and umbilical clamping are common (Northington, 2006). Other methods used are new born asphyxia or carotid artery ligation. These methods have the advantage of not needing anesthesia of the pregnant mothers (Domoki, et al., 2010).

Sheep are more analogous to the human with respect to number of fetuses per pregnancy (1-2), whereas pig litter sizes number from 8-14. Although this is less analogous to humans, large litters reduce costs and therefore can result in more statistically powerful studies. Physiologically, these animals are a good intermediate between rodent studies and non-human primate studies.

There are many limitations of these large animal studies. High costs of care are an issue as well as long developmental and gestational periods. This makes large experiments difficult, and limits the statistical power of studies that involve these animals. Furthermore, the behavior of on these animals is poorly characterised compare with rodents or humans, and the animals develop more quickly than humans, making long term comparisons difficult. Despite this, these models are popular as there are fewer ethical issues compared with primates, and the animals are readily available due to agricultural production.

4.3. Rat and Mouse

Rat and mouse models are the most popular models used in HI. The use of these animals has several advantages. These animals have fast development, are small in size, and are cheap to purchase and maintain, these factors allow for high numbers in a study. This increases the power of the study allowing researchers to study putative protective therapies that may only produce a small improvement in the condition. Furthermore, researchers can study many potential therapies in a relatively short period.

The availability of genetic knockouts and specific phenotypic strains are a further advantage of rodent models. Genetic knockouts allow for the testing of the function of certain genes/proteins during and after HI, as well as facilitating the testing of the specificity of probes used to assay changes in proteins (Chen, et al., 2006). Specific phenotypes, such as spontaneously hypertensive rats, allow the study of co-morbidities which can be common in human conditions (Bloch, 2005).

Using histological analysis such as cellular layering, myelination and neurogenesis, it appears that the development stage that is equivalent to the human at birth is after birth in the rodent is approximately 7 days post birth in both mice and rats (Ten, Bradley-Moore, Gingrich, Stark, and Pinsky, 2003; Vannucci and Vannucci, 2005). Although age correlations differ depending on which histological, behavioral, or physiological aspect is compared (Rice and Barone, 2000). This means the injuries can be induced in pups, without complicated caesarian. Although, some umbilical clamping models are still used in rodent models, by the far the most common models involved ligation of one or more of the blood vessels that supply brain 7 days after birth. Two main models exist: (i.) ligation of both common carotid arteries, leaving only the vertebral arteries to supply blood to the brain. This results in global bilateral damage to the cortex. (ii.) A more common method involves ligation of one of the common carotid arteries followed by a period of hypoxia normally facilitated through a chamber filled with 5-8% oxygen in nitrogen. 7 day old rats normally require 2-3 hours of hypoxia to acutely produce a large unilateral infarction of the cerebrum, mice and older rats require much less time to produce a similar infarct (40-70 mins) (Jack R. Rivers, et al., 2011). The advantage of the latter model is that it leaves an intact hemisphere which allows for histological, biochemical and behavioral comparisons. However, recent evidence now

suggests that the apparent healthy hemisphere may have low levels of damage (Huang, Liu, Cheung, and Chen, 2009).

The shortfalls of rodent model arise from the difference in anatomy and physiology compare with humans. The development of the rodent brain is faster, with adult-like behavioral phenotype reached 20 days after birth in rats. There is therefore a clear gap between these models and clinical applicability. Criticism of preclinical research have increased steadily over the last two decades as therapies found to be effective in preclinical research are failing to produce similar effects in large and expensive phase three clinical trials (Wahlgren and Ahmed, 2004). Some of these criticism focus on the models themselves, questioning the use of models such as rodent ones and whether they produce similar pathological physiological states to those in human conditions (Wahlgren and Ahmed, 2004).

5. TREATMENTS

5.1. Current Clinical Treatments

5.1.1. *Prevention: Pharmacological and Surgical Interventions*

Advances in prenatal and birthing care and diagnostics has seen significant decreases in perinatal HI and improved outcomes after HI (Smith, et al., 2000). Detection of complications such as nuchal cord and maternal infections has increased with greater monitoring during pregnancy. This monitoring includes the use of cardiotocography to monitor the heart rate of the fetus, thus detecting possible causes of global hypoxia such as defibrillation or pathological bradycardia. Also, the use of fetal imaging using color Doppler and ultrasound, can detect if a complication like a tight nuchal cord requires more intense monitoring and possible cesarean birth (Ogueh, et al., 2006).

Oxytocin is a hormone that causes labour induction, and can be administered as a drug to induce labour (Milson, et al., 2002). Inducing labour has lowered the prevalence of HI by inducing birth in cases where the fetus has an acute complication such as bradycardia. Also if the pregnancy has gone on beyond 42 weeks, the size of the fetus increases the risk of birthing complications that are associated with hypoxia during birth (Ogueh, et al., 2006).

5.1.2. *Rehabilitation: Physiotherapy*

Neuroplasticity is the ability of the brain to remodel structurally and functionally. The primary mechanisms of neuroplasticity is changes in the processes of the neurons including dendritic and axonal branching, changes in synapse number, and the formation of new neurons through neurogenesis (Holt and Mikati, 2011). After HI there is a neuroplastic reorganisation process that redistributes function from the damaged areas of the brain to the healthy cortex. In peri/neo natal HI dendritic and axonal branching as well as synaptogenesis is most likely the most important mechanism of neuroplasticity, as in the developing brain a surplus of neurons already exists (Holt and Mikati, 2011).

Neuroplasticity is strongly influenced by activity of the cortex. Pathways that are used and therefore activated more frequently are strengthened and developed. The developing brain is particularly plastic and is therefore better able to recover from injury. Therapies have been successfully developed for after HI that assist the rewiring process that is needed to

maintain normal function. Challenging the affected individual in cognitive and motor tasks is referred to as enrichment, and has been shown to assist in the rewiring process.

Therapies involving increasing the movement of the effected limbs have been shown to be successful in adult stroke, and there is growing evidence that this is also true for peri/neo-natal HI. From stroke studies it appears that several methods are useful, these include the manual assisting of movement of the limbs by physiotherapist in severally disable individuals. Also, constraining the use of unaffected limbs in more able individuals encourages use of the disabled limb. In the only slightly impaired individuals with mild dyspraxia, challenging games can be used, including ball games and precision grip games, to improve daily motor function (Kolk, et al., 2010).

Transcranial magnetic stimulation (TMS) is a strong directional magnetic field applied to the surface of the skin above the target area that induces depolarization of the neurons under the area. When applied at different intensities and frequencies the brain region can be either induced into a hyper-excitabile or a hypo-excitabile state. This is thought to occur through long term potentiation (LTP) and long term depression (LTD) respectively (Holt and Mikati, 2011; Thordstein, Hallbook, Lundgren, van Westen, and Elam, 2011). TMS they can downregulate or upregulate the use of certain brain regions, and so might be used to increase the functionality of a damaged area by facilitating neuroplasticity, including by temporarily arresting function in complementary undamaged brain regions (thereby forcing use of compromised brain regions) (Bernard, Goldenberg, Armstrong-Wells, Amlie-Lefond, and Fullerton, 2008).

5.1.3. Limiting Brain Damage: Pharmacological Intervention

There is no pharmacological intervention for use after peri/neo-natal HI that has completed stage 3 clinical trials (Scafidi and Gallo, 2008; Yager, Armstrong, and Black, 2009). Current pharmacological interventions involve tightly controlling the homeostasis of the infant. The two systems that are most crucial to the infant survival and that must be controlled are carbon dioxide and blood glucose homeostasis. Hypocapnia or low CO₂ levels of the blood are associated with worsened outcomes following HI. Low levels of CO₂ results in vasoconstriction, potentially further reducing oxygenated blood flow to the brain, and increasing brain damage. However, despite excellent preclinical evidence, no clinical evidence exists that maintaining high of CO₂ is neuroprotective. In spite of this it still seems prudent to maintain normal or high levels of CO₂ of ventilated infants following HI. With respect to blood glucose, hyperglycemia has been shown in animal models to be neuroprotective, yet no clinical data exists on glucose related interventions. Hypoglycemia has been associated with poorer outcomes following HI and thus glucose levels are tightly controlled.

Seizure activity is common in the first 72 hours after an HI incident, and rarely afterwards. These seizures are not controlled by anticonvulsants as the potential risks of administering anticonvulsants to the developing brain may outweigh the potential benefits. A metaanalysis of 7 different placebo controlled clinical trials that use anticonvulsants showed no benefit to the clinical outcomes of the infants (Evans, Levene, and Tsakmakis, 2007). Furthermore, seizures may be a symptom of mass neuronal death and may not be involved in exacerbating the damage (Jack R. Rivers, et al., 2011). However, seizures have been associated with an increase in body temperature that will worsening the pathology (Wirrell, Armstrong, Osman, and Yager, 2001). Conversely HI induced epilepsy that continues beyond

infancy must be controlled with antiepileptic to limit continued damage and improve quality of life (Glass and Sullivan, 2009).

5.1.4. Limiting Brain Damage: Hypothermia

Hypothermia can be induced by placing the infant in cold water, or by use of a cold cap on the infants head. Both methods are used to lower the body temperature of the newborn by 3–4°C. Many mechanisms of neuroprotection have been proposed.

Hypoxia reduces rates of reactions and thereby lowers the metabolic demands of cells within the brain; this allows them to operate on a low oxygen and glucose budget. Animal model studies have shown a complete preservation of ATP levels from hypothermia (Yager and Asselin, 1996). This would prevent the loss of function of crucial cellular functions such as Na⁺/K⁺ ATPase and thus maintain membrane potential, preventing excitotoxic cascades and ROS/RNS related damage. The second mechanism could be an immune cell modulation effect. Immune cell function and reactivity is lower in lower temperatures, this could prevent the pathological neuroinflammatory effect seen for days or weeks after HI (Maekawa, et al., 2002). This mechanism could also explain why hypothermia must be maintained in the clinic for the relatively large period of time of 72 hours in order to be effective.

5.2. Putative Pharmacological Treatments

Apart from hypothermia, physical rehabilitation, and preventative surgical and pharmacological intervention, there have been few if any drug treatments that have been proven to be effective at reducing the burden of disability due to neonatal hypoxic encephalopathy. Nonetheless the pharmacological management of peri/neonatal hypoxia is an area of intense research. Treatment strategies can be roughly divided into those that are intended to reduce brain damage either during or subsequent to hypoxia ischemia, and those that are aids to rehabilitation.

5.2.1. Limiting Brain Damage

The pharmacological strategies for reducing brain damage following hypoxia ischemia that have been most intensively investigated can be grouped according to the main elements of the pathological cascade described above; excitotoxicity, oxidative stress, and inflammation. These correspond to drugs that reduce CNS excitation, antioxidant therapies, and anti-inflammatories and other drugs that affect wound healing respectively.

5.2.1.1. Inhibitors of CNS Excitation

Excitotoxicity is fundamentally a property of excessive glutamate signaling, and so antagonists at glutamate receptors have been at the forefront of attempts to reduce brain damage. These include inhibitors at the glutamate NMDA and AMPA receptors. NMDA receptor inhibitors have been extremely successful in a variety of animal model of cerebral ischemia, often causing dramatic reductions in brain damage (Clarkson, et al., 2005; Fernandez-Lopez, et al., 2006). However, this success in preclinical research has not translated into successful treatments in the clinic (Hoyte, Kaur, and Buchan, 2004; Lo, 2008a).

The reasons for this continue to be debated, with quality of preclinical experiments and publication bias cited as a factor (O'Collins, et al., 2011; E. Sena, van der Worp, Howells, and Macleod, 2007; E. S. Sena, van der Worp, Bath, Howells, and Macleod, 2010) along with the inapplicability of animal models and the incompatibility of the time windows for therapeutic intervention with those used in animal testing. The timing of intervention appears to be critically important; NMDA receptor antagonists are protective in the acute stages of injury, but interfere with the beneficial effects of NMDA receptor activation that occur after injury during recovery and repair processes (Lo, 2008b).

Other drugs such as cannabinoid CB1 receptor agonists have been tested as neuroprotectants, with mixed results. Although CB1 receptors expressed on glutamatergic neurons can play a protective role by inhibiting glutamate release, CB1 can also be expressed on GABAergic neurons, and have an excitatory effect by inhibiting GABA release. As a result, cannabinoid CB1 agonists have given mixed results for neuroprotection, with no drugs in development. Similarly, CB1 receptor antagonists are protective in some models, but translation to the clinic does not seem likely at the time of writing (Ashton and Glass, 2008).

Despite the narrow time window for beneficial results from the administration of glutamate receptor antagonists and the problems with delayed administration of NMDA receptor antagonists, other glutamate receptors may still be a target for pharmacological management of hypoxic encephalopathy. Neonatal hypoxia is associated with seizures, which may either be a cause or a consequence of encephalopathy. This has suggested suppression of neuronal excitation as a treatment to reduce the long term effects of neonatal hypoxia. There are many possible targets for intervention, including most of the traditional targets for anticonvulsant and antiepileptic therapy, including glutamate receptors, GABA receptors and voltage sensitive sodium channels. Rat studies have indicated that inhibition of excitatory amino acid neurotransmission may be better in this respect than agonists at inhibitory receptors, such as benzodiazapenes (Jensen, 1995).

Talampanel (GYKI53773) is a non-competitive AMPA receptor antagonist (Aujla, Fetell, and Jensen, 2009) which has been shown to reduce seizures in a dose-dependent manner in a rat model of neonatal hypoxia. Given that seizures are predictive of brain damage in hypoxia ischemia (Jack R. Rivers, et al., 2011) then talampanel could mark a change in strategy for the pharmacological management of CNS excitation in neonatal hypoxia, shifting from neuroprotection in the acute phase to the reduction of seizure-induced injury in the delayed phase of damage. Talampanel is under trial as antiepileptic drug in children and so may be used in the near future in the treatment of seizures associated with neonatal hypoxic encephalopathy. There is also some evidence of neonatal seizures can be successfully treated with traditional CNS depressants such as short acting benzodiazapenes (Sirsi, Nangia, LaMothe, Kosofsky, and Solomon, 2008). As there is considerable overlap between management of epilepsy subsequent to hypoxic encephalopathy and the reduction of seizure induced brain damage, then despite their failure as neuroprotectants in the acute phase of injury CNS inhibitors are likely to play a significant role in the pharmacological management of hypoxia ischemia.

5.2.1.2. Antioxidants

Drugs that target oxidative stress by ROS or RNS, antioxidants, like NMDA receptor antagonists, provide significant neuroprotection in animal models (Clarkson, et al., 2004; Gitto, Pellegrino, Gitto, Barberi, and Reiter, 2009; Sutherland, et al., 2004). However, also

like NMDA receptor antagonists, these drugs have failed to work in the clinic. To take one well discussed example, NXY-059 is a free radical spin trap that unexpectedly failed in clinical trial in the SAINT-2 trial (Shuaib, et al., 2007).

Aside from the issue of the applicability of animal models to humans, there are at least three major arguments for why anti-oxidants have failed in clinical trial. First, cells contain a battery of endogeneous antioxidant mechanisms, such that oxidative stress is the result of an imbalance between free radical and ROS production and endogenous protective mechanisms. Therefore, the concentration of exogenous antioxidants to the sites of injury has to provide at least as much protection as that provided by endogenous mechanisms to be of benefit. The delivery of antioxidant drugs to potential sites of action has been challenging, and has been the topic of a great deal of innovative and ingenious research. Despite this, there is a large experimental gap between the behaviour of antioxidants *in vitro* and their effects *in vivo*.

However, this does not explain why antioxidants have been successfully used in animal models, and so some researchers have argued that exogenous antioxidants do reach key sites of action in significant quantities, but have complex effects depending upon subtleties of timing, dose, and physiology. Reactive oxygen and nitrogen species are not only causes of damage at high concentrations, but are also signaling molecules in various aspects of physiology and homeostasis; for example, the maintenance of blood-flow. Moreover, sustained antioxidant treatment might cause adaptive changes in endogenous protective mechanisms, with unpredictable effects. And in a similar way to NMDA antagonists, although blockade of oxidative stress may be protective in a short time window of acute injury, antioxidants may have biphasic effects such that delayed administration interferes with essential recovery and repair processes (Lo, 2008b).

A third argument maintains that antioxidant therapy may well remain a viable treatment strategy for neuroprotection, but preclinical research has been of insufficient quality to support the decisions that have been made to move drugs into clinical trial. Macleod et al. (Macleod, et al., 2008) have argued persuasively that the pre-clinical evidence for NXY-059 did not warrant progress to clinical trial, and that close analysis of the preclinical studies reveals a paucity of evidence for the drug's efficacy, with effects overestimated due to a lack of rigor in experimental design. The authors argue that this could be corrected by the widespread adoption of publication standards for preclinical animal studies similar to those that are now standard for clinical trials.

5.2.1.3. Anti-Inflammatory Drugs

If it is true that putative neuroprotectants necessarily have biphasic or even multiphasic effects dependent upon timing of delivery with respect to the phase of injury and recovery (Lo, 2008b), then this applies equally to anti-inflammatories. But unlike NMDA receptor antagonists and antioxidants, anti-inflammatories tend to have beneficial actions in the delayed phase of injury, whereas delivery at the acute phase can interfere with vital triggers to endogenous protective and recovery mechanisms.

The two most commonly used classes of anti-inflammatory drugs are corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs). Neither of these has proven suitable for treatment of cerebral ischemic injury. NSAIDs have strong effects on haemostasis; traditional NSAIDs increase risk of hemorrhage, and selective COX-2 inhibitors increase the risk of thrombosis. It is not clear as yet whether these limitations will apply to all drugs that target the arachidonic acid pathways such as leukotriene inhibitors. With respect to steroids, not

only is there no evidence for any beneficial effect the acute phase of injury (De Reuck, et al., 1988; Norris and Hachinski, 1986) but various lines of evidence suggest that high dose steroid treatment can cause microvascular injury and vasospasm (De Reuck, et al., 1988; Edlow, et al., 2010; Linn, Desilva, and Peeters-Asdourian, 2009). Because of this, testing of non-traditional anti-inflammatory drugs in animal models of cerebral ischemia is an active field of research. For example, minocycline is neuroprotective in a range of models, including hypoxia ischemia (Cai, Lin, Fan, Pang, and Rhodes, 2006; Familian, Boshuizen, Eikelenboom, and Veerhuis, 2006; Maier, et al., 2007).

Another example of a non-traditional anti-inflammatory target is the cannabinoid CB2 receptor (J. R. Rivers and Ashton, 2010b). Agonists for the CB2 receptor have been shown to be potentially neuroprotective in cerebral ischemia (Zhang, et al., 2007) although it has not yet been determined whether this is due to an anti-inflammatory effect, and whether CB2 activation is neuroprotective in the secondary phase of cerebral ischemic injury.

5.2.2. Rehabilitation and Repair

Just as non-pharmacological interventions have focused both on limiting brain damage during injury and on enhancing recovery of function through rehabilitation programs, so pharmacological strategies include not only neuroprotective drugs but also drugs that may act in a way to facilitate the plasticity of neural and associated tissue that is central to repair and recovery.

5.2.2.1. Neural Plasticity

The emerging evidence that drugs that are neuroprotective in the acute phase of injury can be damaging in the secondary phase (and vice versa) highlights the importance of the recovery and repair processes that take place after acute injury. The secondary phase of injury involves not only ongoing cell death, but also changes in microvasculature due to angiogenesis, neuronal remodeling such as that due to axonal sprouting, complex patterns of neuronal arrest and recovery due to endogenous inhibitory mechanisms, and more controversially, the generation of new cell cells from neural progenitors. A highly orchestrated and complex inter-related series of events takes place in the hours, days and months following cerebral injury, and only recently has it become clear that processes that are damaging over the short term in one phase of injury may provide vital triggers for recovery processes that follow (Lo, 2008b). To give three examples: (i.) matrix metalloproteinase (MMP) inhibitors are protective in the acute phase of injury by stabilizing the microvasculature, but are vital for vascular remodeling and angiogenesis in the recovery phase (S. R. Lee, et al., 2006); (ii.) activated microglia and macrophages may be cytotoxic in one phase of injury, but through phagocytosis perform a vital role in preparing tissue for subsequent axonal sprouting and remodeling; (iii.) although over-activation of NMDA-receptors leads to excitotoxicity in the acute phase of injury (above), functioning NMDA receptors appear to be vital for not only the activation of endogenous neuronal protective mechanisms (Papadia, Stevenson, Hardingham, Bading, and Hardingham, 2005) but are also a central element in the regulation of the synaptic plasticity that is critical for the remodeling of neural networks in the recovery phase.

Considerations about the timing of delivery of protective drugs aside, pharmacological strategies for enhanced neural plasticity are still in the conceptual stage. Identification of biochemical targets for increased plasticity is a goal, but to our knowledge not yet an

established reality. Neurotrophic factors, neurosteroids, and glutamate receptor modulators are just some examples. However, one promising approach has been suggested by recent research by Clarkson et al. (Clarkson, Huang, Macisaac, Mody, and Carmichael, 2010) who used an inhibitor of GABA transport to enhance motor function in mice following stroke. GABA transporter dysfunction is thought to be a contributing element to the tonic inhibition of neural activity in the penumbral regions of cerebral injury, such that substantial neural capacity may not be truly lost, but be simply in a state of arrest. Disinhibition of these arrested neurons in the delayed phase of injury may not only provide some immediate recovery of function, but by restoring normal neural activity allow for ongoing neural plasticity. An optimal strategy for the use of such drugs would seem to be as adjuvant to physical therapy, with drug treatment allowing for the neural activity necessary for the plastic changes guided by a rehabilitation program. Ideally, drugs will be found to enhance neural plasticity such that the effects of physical rehabilitation may be even further accelerated and amplified.

5.2.2.2. Neurogenesis

Since the discovery that there are two areas of the brain that produce new neurons throughout adult life (the sub-granular layer in the hippocampus and the sub-ventricular layer in the striatum) there has been hope that this may be exploited to improve recovery from injury (Okano and Sawamoto, 2008). However, although it seems clear that neurogenesis contributes to neural plasticity in the hippocampus and the olfactory bulb in healthy adults, it has not yet been determined whether the production of new neurons in the pathological brain is part of a recovery process (Vert and Daval, 2006). Neurogenesis is upregulated in a variety of neural pathologies, including chronic neurodegenerative diseases such as Huntington's disease (Vazey and Connor, 2010) and following acute injury such as stroke (Kozorovitskiy and Gould, 2003). Whether this is part of an endogenous repair process or merely an epiphenomenon of disorganized cell division, remains to be determined (Minger, et al., 2007; Zhao, Deng, and Gage, 2008).

Cells produced at the sub-ventricular layer do migrate to sites of injury following cerebral ischemia, but it has been estimated that only a small percentage of these differentiate into neurons (Vazey and Connor, 2010). Of the new neurons produced, it is not known whether they integrate into functioning neural networks and thus contribute to recovery. Following asphyxia in rats, cell proliferation is increased in the hippocampus, but most of these cells are glia (Keilhoff, John, Langnaese, Schweizer, and Ebmeyer, 2010). Therefore, instead of using one or more of the numerous drugs that are known to increase neurogenesis (Jagasia, Song, Gage, and Lie, 2006), a more nuanced treatment strategy would be to alter the microenvironment of the injured regions of the brain in such a way that facilitates cell survival, differentiation, and integration. Due to the potential impact this could have in providing more extensive neural networks for adaptive recovery, this is another field of intense research activity. By contrast, although pharmacologically tractable, it is not yet known whether increasing the rate of cell division and migration from the neurogenic zones of the brain could be hazardous. For example, it is not known whether neurogenesis is an adaptive response to epilepsy, or whether it is a causal factor (Bernardino, Ferreira, Cristovao, Sales, and Malva, 2005). A challenge for neurogenesis research in hypoxia ischemia therefore is the identification of targets for improvements in adaptive and not maladaptive plasticity.

CONCLUSIONS

Hypoxia ischemia of the neonate is a common problem that reduces quality of life and can be fatal. HI causes a range of symptoms that include cognitive impairment and motor dysfunction, which can be debilitating for a lifetime. Following the period of low oxygen and low nutrients, regular cellular processes are impaired resulting in an excitotoxic and ROS/RNS cascade that is highly toxic to both neurons and oligodendrocytes progenitor cells. Subsequent damage is followed by a long period of neuroinflammation that lasts for weeks and evens months after the initial insults and causes further damage through cytokine signaling as well as excitotoxicity and RNS/ROS compounds. Despite increasing knowledge of the details of these processes, no pharmacological interventions are currently in clinical use, and hypothermia is currently the only intervention used to prevent brain damage. A variety of animal models have been developed to study this pathology but treatments found to be efficacious in these models have not translated into the clinic. This suggests that a change in the way animal models are used is needed. When putative treatments are tested, several different species and both sexes should be used, the treatment should be administered at a clinically relevant time after neurological insult, animals should be assessed over a prolonged period of time including behavioral tests, and co-morbidities should be addressed. The latter include preterm birth, hypocapnia, hypoglycemia and, possibly, maternal infections. These shifts may already be happening, and treatments may yet emerge from existing knowledge of mechanisms of neuroprotection. Strategies that should not yet be dismissed include anti-inflammatory treatment, inhibition of CNS excitation, antioxidant therapy, and facilitated neuroplasticity. The challenge now for researchers is to determine optimal times, doses, and regimes for maximizing the potential benefits of these treatments whilst minimizing the risks.

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Chapter 42

SWALLOW SCREENING AS AN ESSENTIAL COMPONENT OF ACUTE STROKE MANAGEMENT

Julie Luker and Kylie Wall

ABSTRACT

Dysphagia following stroke is common and increases patient risk of poor outcomes and adverse events. Swallow screening is an important process of care for patients who suffer acute stroke. This brief examination aims to identify those patients that may be at risk of swallowing problems so that comprehensive assessment and management can be provided. However, despite wide agreement that swallow screening is an essential component of care, there is no agreement regarding how or when screening should be performed, and by whom. Debate continues regarding the validity and reliability of various screening tools, and whether they are able to provide an acceptable level of certainty in risk detection. Our chapter reviews the current evidence underpinning swallow screening for acute stroke patients.

Published clinical quality audits on acute stroke populations have reported suboptimal compliance with swallow screening. Current evidence suggests that multiple factors contribute to screening compliance, including the structures and systems within health care facilities, such as stroke unit care. The provision of swallow screening may also be influenced by demographic and stroke-related variables of the patients themselves, such age, gender and stroke severity.

The results of the authors' recent research on the factors that influence the quality of stroke swallow screening will be presented. This was conducted in three Australian tertiary hospitals. The study retrospectively audited the care provided to 300 patients admitted with acute stroke. Data was collected on swallow screen compliance and other variables of interest including stroke unit admission, day of admission, patients' age, gender, English proficiency, comorbidities, pre-stroke levels of independence and type of accommodation, stroke severity and length of stay. Logistical univariate and multivariate stepwise regression analysis was undertaken to explore the determinants of screening compliance in this population. In the sample, patients with milder stroke, and those admitted over a weekend, were at increased risk of not receiving a swallow screen.

Swallow screening is an accepted care process in acute stroke management, despite ongoing debate regarding the best way to conduct the screening. Studies, including our own, report suboptimal provision of swallow screening. The factors that influence

swallow screening compliance may be complex and include variables within hospital systems, as well as variables related to the patient and their stroke.

INTRODUCTION

Stroke is a major health issue internationally. In Australia alone around 60,000 people suffer stroke annually making it the country's second greatest cause of death and the leading cause of disability (1, 2). The past decade has seen an increase in research evidence regarding the best care for acute stroke patients, aimed at optimising their outcomes. This has led to the development of high quality stroke guidelines to steer clinical care (3). Guideline recommendations encompass the prevention of complications associated with stroke-related mortality and other poor outcomes (4).

Oropharyngeal dysphagia is a common consequence of stroke and it increases patient risk of post-stroke complications. Reported incidence of dysphagia varies, with an Australian national audit reporting 47% (5), and instrumental videofluoroscopic examination reporting that dysphagia affects 64 – 78% of stroke patients (6). About half of these patients with dysphagia will recover a functional swallow within a week, but the remainder may experience dysphagia for several months or longer (6, 7). A systematic review and meta-analysis conducted by Martino's group (6) confirmed that patients presenting with dysphagia had a significantly increased risk of developing pneumonia (RR 3.17; 95% CI 3.3-39.7). Dysphagia is also associated with increased risk of malnutrition and dehydration, increased length of stay and in-hospital death (6, 8, 9). Given the serious potential consequences of dysphagia, it is unsurprising that the detection, assessment and management of this condition is prominent in stroke guidelines.

The provision of routine swallow screening had been shown to improve patient outcomes and reduce the occurrence of pneumonia following acute stroke (10, 11). However, despite wide agreement that swallow screening is an essential component of care, there is no agreement regarding how or when screening should be performed, and by whom.

In this chapter, swallow screening is defined as a brief examination of swallow function which aims to detect patients who may be at risk of swallowing problems so that comprehensive assessment and management can be provided. Patients found to have a safe swallow at screening, can commence oral intake without the delay of waiting for a specialist assessment (12). Screening is often administered by non-specialists in dysphagia including nursing staff, or any other member of the multi-disciplinary team. Ideally, screening requires minimal training to educate the screening staff on the basic understanding of dysphagia and administration of the screening tool.

In this chapter screening is differentiated from a comprehensive bedside swallow assessment, which is defined as a more detailed procedure performed by clinicians with specialised knowledge in dysphagia and skills in interpreting findings, such as a speech pathologist.

WHEN TO SCREEN AND BY WHOM?

We recently conducted a systematic review on the use of process indicators to determine the quality of care provided to patients with dysphagia following acute stroke (13). All 21 reviewed articles used process indicators for swallow screening as a measure of quality stroke care. However there was no agreement regarding the optimal timing of screening: seven articles specified screening performed before oral intake, seven used a timeframe of within 24 hours of admission, while seven authors did not mention timing as a quality-of-care consideration. As most of the reviewed articles were retrospective studies, it is possible that this equivocal evidence on the best time to screen reflected pragmatic difficulties of collecting retrospective data on oral intake, rather than sound evidence of effectiveness. *A priori* reasoning would suggest that oral intake should be forestalled until a safe swallow is confirmed, and this is in fact reflected in recent clinical guideline recommendations (3)(p82).

There are no current evidence-based recommendations for the level of training required to perform a swallow screen. Australia's Clinical Guidelines for Stroke Management simply specify that "Personnel specifically trained in swallow screening using a validated tool should undertake screening" (3)(p82). Speech Pathologists or Speech & Language Therapists (SPs) are recognised experts in the assessment and management of dysphagia (14). However, given the requirement for rapid swallow screening for all stroke admissions over seven days a week, allocating this role to SPs is an inefficient use of resources (12). Emerging evidence suggests that training nursing staff to conduct swallow screening is a feasible option. Cichero's study (12) with stroke and non-stroke patients reported that nurses who undertook a detailed training program conducted by SPs were able to adequately detect the risk of dysphagia on screening. Compared to repeated screening conducted by SPs, nurse-screening had 95% sensitivity and 97% specificity. This is supported by the Weinhardt et al. (15) study, where 94% agreement was demonstrated in swallow screen results when conducted by registered nurses and SP staff with a population of acute stroke patients.

SCREENING TOOLS AVAILABLE

Debate continues regarding the validity and reliability of various screening tools and whether they are able to provide an acceptable level of certainty in dysphagia detection. Current stroke clinical guidelines have been unable to make many specific recommendations due to a lack of clarity in the underpinning evidence (3, 6). A large number of studies have attempted to establish the most valuable screening tools, however flawed methodological quality and variability between studies has failed to lead to a unanimous conclusion (6, 10).

The accuracy of swallow screening in detecting stroke related dysphagia is particularly difficult for the proportion of patients who exhibit no overt signs of aspiration, such as coughing. This silent aspiration has been reported in 28-52% of acute stroke patients (16). The Nakajoh et al. (2000) study on stroke patients revealed that the latency of the cough reflex was significantly associated with the incidence of pneumonia (17). Therefore screening tools that depend on an overt cough response to penetration and/or aspiration may miss many stroke patients who are at risk.

Variable information is available on the psychometric properties of some swallow screening tools. Determining validity, sensitivity and specificity relies on the ability to compare findings from screening tools to a recognised ‘gold standard’ however agreement on a reference standard for detecting post-stroke aspiration has been problematic (18). While videofluoroscopy (VFS) has been long considered the reference standard for swallow assessment (19), this is not unanimously accepted for acute stroke patients (18). Problems exist with the feasibility of using VFS following stroke, especially for severely affected patients with poor sitting balance. Furthermore, the inter-rater reliability data on VFS to detect dysphagia is also poor (20, 21), and evidence is still emerging regarding the best materials and measurement tools to use during testing (22, 23) .. Fiberoptic endoscopic evaluation of swallow (FEES) is an alternative reference standard for swallowing screening, and FEES has demonstrated sensitivity and specificity values which are comparable to VFS. Although it is an invasive procedure, FEES may be more feasible for use in acute stroke due to bedside portability and reduced radiation exposure (24). Whether using FEES or VFS as the reference test, recent validation studies use penetration to the level of vocal folds and/or aspiration as the end point of testing (25).

Intermittent systematic reviews have tracked the availability of new swallow screening tools over time (18, 25-27). The following is a summary of screening tools which are freely available. Tools were only included where data were available from stroke population studies, and where validity was assessed against the reference tests of FEES or VFS.

Recommended swallow screens that met these criteria were:

- 50ml (3oz) or 90ml (3oz) Water Swallow Test (28-31),
- Gugging Swallow Screen (GUSS) (32)

Table 1 details the psychometric information available for these screening tools. The sensitivity measures indicate the ability of the tool to detect ‘risk’ when it is present, while specificity indicates the tool’s ability to produce negative results for patients who are not ‘at risk’ (27).

A simple pulse oximetry test, in conjunction with 50ml Water Swallow Test, is reported to increase sensitivity and specificity (28, 30) as detailed in Table 2.

The swallow assessment or screening tools which were excluded from our recommendations are detailed in Table 3. The majority of exclusions (seven tools) were due to insufficient validation of the tool, four were considered to require expert interpretation of findings and were therefore unsuitable for inter-disciplinary screening, and one tool was not freely available. Despite these short comings, this information provides a comprehensive over-view of published resources.

Table 1. Psychometric properties of available swallow screening tools.

Test	First author	Sensitivity %	Specificity %	PPV %	NPV %	Reference test	Reliability tested	Stroke sample size
90ml (3 oz) Water Swallow Test	DePippo et al. (29)	76	59			VFS	No	20
	Suiter & Leder (31)	92.7 - 100	40.7 – 54.8	28.4 – 33.3	95.7 - 100	FEES	No	468
50ml Water Swallow Test	Lim et al. (30)	84.6	75	78.6	81.8	FEES	No	50
	Chong et al. (28)	79.4	62.5	81.8	58.8	FEES	No	50
Gugging Swallow Screen (GUSS)	Trapl et al. (32)	100	50 - 69	74 - 81	100	FEES	IRR between therapists (K=0.835; P<0.001)	50

PPV %= Positive predictive value; NPV %= Negative predictive value; IRR= Inter-rater reliability

Table 2. Psychometric properties of oxygen saturation tests to detect dysphagia.

Test	First author	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %	Reference test	Stroke sample size
Pulse oximetry (desaturation $\geq$ 2%)	Lim et al. (30)	76.9	83.3	83.3	76.9	FEES	50
	Chong et al. (28)	55.9	100	100	51.6	FEES	50
Combined 50ml water swallow + pulse oximetry (desaturation $\geq$ 2%)	Lim et al. (30)	100	70.8	78.8	100	FEES	50
	Chong et al. (28)	94.1	62.5	84.2	83.3	FEES	50

Table 3. Swallow screens and assessments not meeting inclusion criteria.

Test	First author	Sensitivity%	Specificity%	PPV %	NPV %	Reason for exclusion
Any Two test	Daniels et al. (33)	92	67			Expert interpretation of findings needed
	Leder et al, 2002 (16)	86	30	50	73	Expert interpretation of findings needed
Bedside Swallow Assessment (BSA)	Smithard et al. (21, 34)	47 -70	66 - 86	50	85	Expert interpretation of findings needed
Burke Dysphagia Screening Test (BDST)	DePippo, (35)	88	22			Only validated against pneumonia as reference test. Incorporates 3oz water swallow test which is validated.
Examination Ability to Swallow (EATS)	Courtney & Flier (36)					No validation data available
Mann Assessment of Swallowing Ability (MASA)	Mann et al. (37)	73	89			Expert interpretation of findings needed
Massey Bedside Swallowing Screen	Massey & Jedlicka (38)	100	100			Only validated against clinical records of dysphagia symptoms as reference test.
Nursing Admission Dysphagia Screening Assessment	Bravata et al. (39)	29	84	50	68	Only validated against NIH Stroke Score as reference test.
Royal Brisbane & Women's Hospital Dysphagia Screening Tool	Cicherio, 2009 (12)	95	97	92	98	Only validated against SP bedside assessment
Standardised Swallow Assessment (SSA)	Ellul, 1993 (40)					No published validation data
	Perry, 2001 (27)	97	90			Only validated against 'Summative clinical judgement' as reference test

Table 3. (Continued)

Test	First author	Sensitivity%	Specificity%	PPV %	NPV %	Reason for exclusion
Timed Test	Hinds, 1998 (41)	97	69			Only validated against SP assessment & symptoms questionnaire as reference tests.
Toronto Bedside Swallow Screening Test (TOR-BSST)	Martino et al. (6)	96	64		93	Not freely available at time of publication
Water Swallow Test (WST) - voice change finding	Nishiwaki et al. (42)	72	67			Expert interpretation of findings needed

PPV%= Positive predictive value; NPV%= Negative predictive value

SWALLOW SCREENING FOR ALL?

Current stroke clinical guidelines recommend swallow screening or assessment for all patients with acute stroke. The authors' recent systematic review reported on 25 studies which examined the quality of care provided for patients with dysphagia in acute stroke settings (13). The majority of these studies (21) included swallow screening as a process indicator of quality. Studies frequently reported poor compliance with the administration of swallow screening, which varied between settings for unclear reasons. To illustrate, Reeves et al. (2008) (43) reported that only 55.6% of patients across 857 United States hospitals with an acute stroke had received a screen. By comparison the national audit of 96 Australian hospitals reported that 64% of patients were screened within 24 hours of admission (5). Rudd et al. (2007) (44) described differing compliance across 46 United Kingdom hospitals depending on access to stroke unit care (73% compliance) or no stroke unit (66% compliance).

Minimal investigations have been undertaken to explore the reasons why some patients receive evidence-based care such as swallow screening, while others do not. Current evidence suggests that multiple factors contribute to the provision of quality care, including the structures and systems within health care facilities, such as stroke unit care (44, 45). However the provision of swallow screening may also be influenced by demographic and stroke-related variables of the patients themselves, such age, gender and stroke severity. Before quality improvement activities can target barriers to care, a better understanding is needed of which patients are at risk of suboptimal care.

A STUDY OF SWALLOW SCREENING COMPLIANCE

A recent study by the authors explored the determinants of quality care for acute stroke patients (46, 47). One of the indicators of quality care in this study was that patients received a swallow screen within 24 hours of admission to hospital.

Methodology

Ethical approval was gained to conduct a retrospective clinical audit of consecutively sampled acute stroke patients' medical records.

Sample Size

Three hundred medical records were sampled, 100 each from three large metropolitan tertiary hospitals in Adelaide, South Australia, Australia. This sample size was sufficiently powered at $\alpha=0.05$, $\beta=0.8$ for sub-group analysis.

Inclusion and Exclusion Criteria

Patients' medical records were included if they had been consecutively admitted prior to August 31st 2009 with an IDC10 diagnostic code of acute stroke which was confirmed by diagnostic imaging or clinical evidence.

Quality of Care Measures

In an earlier systematic review we determined that *swallow screening performed within 24 hours of admission* was one appropriate measure of quality of care provided in acute stroke settings (13). Compliance with this process indicator became the dependent variable for our investigation into the factors which influence care quality for patients with stroke related dysphagia.

Data

Data were extracted from patient records on the independent predictor variables of patients' age, gender, pre-morbid levels of independence and accommodation type, English proficiency, comorbidity levels (Charlson Comorbidity Index), weekend or weekday admission, stroke unit admission, initial stroke severity (NIH Stroke Scale), length of stay in the acute hospital (LOS), and process indicator compliance. De-identified data from all patient records were manually extracted and entered onto a purpose built MS Excel spreadsheet.

Data Analysis

We undertook a series of analyses using univariate logistic regression models to understand the relationships between the predictor variables and compliance with swallow screening. Details of this analysis, and the way that data were managed, are available elsewhere (46, 47). Correlations between variables were expressed as odds ratios (OR) and 95% confidence intervals (95% CI).

Results

Description of Participants

The mean age of the 300 sampled patients at hospital admission was 74.7 years (Standard Deviation (SD) 13.5, range 18–100 years). The sample was proportionally balanced for gender. The mean (SD) ages for males and females were similar, however a larger proportion of females were in the older age groups with 72% females aged 75 years or older, compared to 53% males. A greater proportion of females suffered a moderate or severe stroke (28%) than males (18%). For the whole sample, there were weak relationships between increasing age and higher comorbidity levels ($r^2 = 0.20$), and increasing stroke severity ($r^2 = 0.21$). The mean length of stay in acute care was 12.5 days (SD 15.6, range 1–98 days).

Process Indicator Compliance

Compliance with the process indicator was poor, with only 51.6% of eligible patients receiving a swallow screen within 24hrs of admission.

Variables associated with Swallow Screening Compliance

Univariate logistic regression modelling determined that three of the 10 predictor variables were significantly associated with swallow screening compliance (see Table 4).

Table 4. Univariate model of independent predictor variables and swallow screening.

Predictor variables	Screening compliance
Age (75+ yrs)	1.02 (0.63 – 1.66)
Gender (female)	0.75 (0.47 – 1.21)
Mild stroke severity (NIHSS $\leq$ 8)	0.37 (0.23 – 0.61)**
High comorbidity (CCI $\geq$ 1)	1.29 (0.77 – 2.15)
Previous Independence	0.90 (0.54 – 1.49)
Previous residential care	1.52 (0.73 – 3.17)
Stroke unit admission	0.99 (0.62 – 1.60)
Weekend admission	3.09 (1.84 – 5.22)**
Length of stay (<12 days)	0.50 (0.30 – 0.82)**
English proficient	1.31 (0.54 – 3.13)
Odds Ratios (95% CIs) ** significant	

Patients with a mild stroke (NIHSS $\leq$ 8) or a shorter LOS (< 12days) were less likely to be screened than patients with more severe strokes, or those with shorter hospital stays. Swallow screens were significantly more likely to occur for patients admitted on a weekday compared to weekend admissions.

Accounting for Confounders

Additional univariate analysis demonstrated complex and potentially confounding statistical relationships between several predictor variables (47). This is depicted in Figure 1.

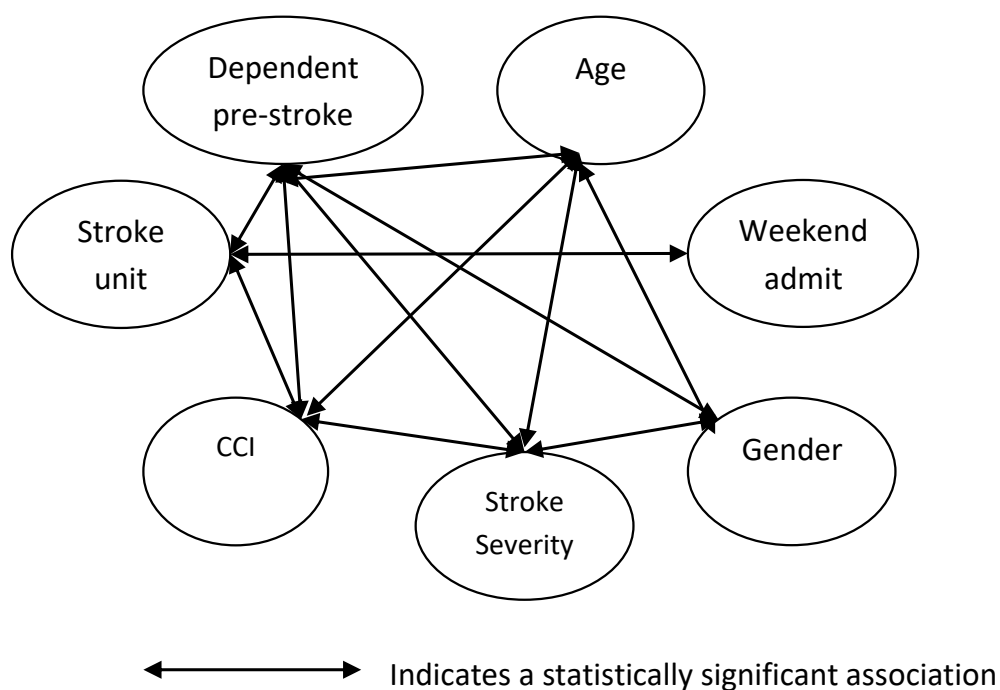


Figure 1. Potentially confounding relationships between predictor variables.

Logistical stepwise multivariate models were used to account for confounders. Only two variables were significantly associated with the provision of swallow screening, after confounders were considered in analysis (Table 5).

Table 5. Variables associated with swallow screening after accounting for confounders.

Predictor variables	Screening compliance	
	Chi ² [Freedom°]	OR (95% CI)
Mild stroke severity (NIHSS≤8)	16.2 [1]	0.4 (0.2-0.6)**
Length of stay (<12 days)	19.0 [2]	0.6 (0.4-1.1)
Weekend admission	36.1 [2]	3.3 (1.9-5.7)**

** statistically significant finding OR= Odds ratio
95%CI= 95% Confidence Interval

Discussion of Study Findings

This study provides new findings regarding the factors which may relate to compliance with swallow screening for acute stroke patients. Our findings support earlier studies reporting the sub-optimal use of swallow screening in practice. The complex associations identified between independent variables indicate that there are unlikely to be simple explanations for why some patients receive recommended dysphagia care and others do not.

We acknowledge that the generalizability of the findings may be limited by some variables chosen in our study, due to international variations in health care policy and systems. Length of stay data and admissions directly to a stroke unit, for example, are both particularly influenced by local contexts.

Our results indicate that patients admitted over a weekend or with mild strokes (NIHSS scores ≤8) may be at particular risk of missing swallow screening. Previous studies have also reported poor quality care for patients admitted over weekends, and have linked this to worse outcomes for these patients (44, 48). The reasons for day-dependent differences in care standards are unclear but are likely to be associated with the reduced availability of trained staff and different operational systems over weekends. It appears that staff are mindful of performing a swallow screen for patients admitted with a severe stroke, perhaps due to more visible cues of risk. However there is no evidence that patients with milder strokes (as determined by NIHSS) are at less risk of dysphagia than patients who have had a more severe stroke. The recommendation of swallow screening for all stroke admissions needs to be more universally implemented.

IMPROVING COMPLIANCE WITH SWALLOW SCREENING

The principles of translating evidence into clinical practice have been well documented by implementation scientists in recent years (49-51). Strategies specific to clinical management in acute stroke settings are emerging and could be applied to the improvement of effective swallow screen processes (11, 12, 52-55). Evidence based strategies that could be considered include: organisational assessment of readiness for practice change, identification of barriers to change, effective involvement of key stakeholders, development of incentives to

change, targeted education programs, and the guided roll-out of the new practices followed by cycles of clinical auditing and feedback.

The research outlined above suggests that there are particular sub-groups of acute stroke patients who may be at increased risk of poor dysphagia management. The swallow screening implementation strategies should ensure that these at-risk groups are carefully considered.

CONCLUSION

Dysphagia is a common consequence of stroke that must be identified early after onset and managed carefully to avoid negative outcomes. Swallow screening for all acute stroke patients is recommended best-practice to efficiently detect those patients at risk of swallowing problems. Debate continues regarding the best way to conduct screening, and screening alone is unlikely to detect silent aspiration. This chapter identified two swallow screening tools that have proven sensitivity and specificity in the acute stroke population. Both these tools can be used by staff from various disciplines, after minimal training. The effective application of swallow screening within clinical settings is challenging. Current evidence suggests that universal swallow screening is not well translated into clinical practice. Some patients may be at greater risk from unidentified dysphagia than others. The results of our recent study demonstrated that patients admitted over a weekend and those with milder strokes were more likely to miss out on timely swallow screening. Acute stroke facilities should consider systematic ways to ensure optimal care for all patients.

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Chapter 43

ANALYSIS OF BRAIN FUNCTION USING CEREBRAL BLOOD FLOW MEASUREMENTS IN PATIENTS WITH SUPRATENTORIAL CEREBRAL STROKES

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ABSTRACT

Background: Cerebral blood flow (CBF) in chronic stage after cerebral stroke is considered to reflect a brain function. All the brain structures mutually influence, however, various territories show specific functions. We investigated territories that strongly affected activities of daily living (ADL) by using Xenon-enhanced computed tomography (Xenon CT).

Methods: A total of 130 patients with an average age of 67 years, including 80 patients with unilateral cerebral hemorrhage and 50 patients with unilateral cerebral infarction, were enrolled in this study. Xenon CT was performed 39 days on average after stroke onset to predict rehabilitation outcomes, and the ADL was evaluated using the functional independence measure (FIM) at hospital discharge, 104 days on average after stroke onset.

Results: 1. In all groups of right/left cerebral infarction and hemorrhage, mean CBF (mCBF) values on average of the lesion side were from 24.7 ml/100g/min to 27.9 ml/100g/min, and significantly decreased with differences of 6.1 ml/100g/min to 3.3 ml/100g/min in comparison with the non-lesion side. 2. Spearman's rank correlation analysis revealed that the FIM total score of right cerebral infarction was significantly correlated to both right ($r_s=0.508$, $p<0.001$) and left ($r_s=0.465$, $p<0.002$) mCBF values, however, that of left cerebral infarction was not correlated to them. 3. According to the subgroup analysis of territories about cortical and perforating arteries, only the group of right cerebral infarction with lesions in cortical artery territories showed significant correlation coefficients between FIM total scores and both right ($r=0.656$, $p<0.001$) and left ($r=0.751$, $p<0.001$) mCBF values. 4. As a common result of right and left cerebral infarction groups, regional CBF (rCBF) values of right frontal cortex and rCBF values of

right thalamus were significantly correlated to FIM motor scores, and rCBF values of right and left putamen were significantly correlated to FIM cognitive scores. 5. Cerebral hemorrhage did not show the same results as cerebral infarction, supposedly due to mass effect of hematoma.

Conclusion: Our results seemed to support the idea that the function of right brain, which plays an important role in spatial recognition, influences better outcomes more than the left-brain. In addition, we proved that the putamen, which controls a various kinds of motor skills, was related to cognitive function.

INTRODUCTION

Depending on brain activities such as motor and cognitive functions, normal brain tissue consumes oxygen and glucose, coupling with cerebral blood flow (CBF), and consequently, the CBF could be an indicator to evaluate brain focal function. However, when a cerebral stroke happens, the CBF begins to alter and shows a discrepancy between clinical symptoms and its value, such as luxury perfusion, which condition is that CBF exceeds the metabolic requirements, and misery perfusion, which condition is that CBF is lacking. During neural stimulation such as somatosensory and visual ones, imbalance of blood flow and oxygen metabolism was also observed [1]. Accordingly, the CBF in acute stage after stroke onset is inappropriate to be a parameter to evaluate the brain function. Nevertheless, in the chronic stage of cerebral stroke, the CBF resumes to agree with both cerebral circulation and functions, and hence the CBF at rest in the chronic stage comes to be a scale to assess the brain function.

There are a variety of modalities to evaluate the CBF and related metabolic values such as positron emission computed tomography (PET), single photon emission computed tomography (SPECT), functional magnetic resonance imaging (MRI) [1], and near infrared spectroscopic topography (NIRS) [2]. Xenon-enhanced computed tomography (Xenon CT) [3], which provides quantitative regional CBF (rCBF) values with a CT scan and a simpler system, is also a useful method to assess the brain activity.

We reported that the CBF in the chronic stage had a significant relation with the activities of daily living (ADL) at the end of stroke rehabilitation using Xenon CT [4]. In the study, we concluded that right mean CBF (mCBF) tended to be more closely correlated with functional independence measure (FIM) total scores at discharge than left mCBF indifferent to the type of stroke. The FIM is a scale to evaluate ADL of patients with a stroke or other neurological disorders from both sides of motor and cognitive function. The pathological features are different among cerebral infarction (CI), intracerebral hemorrhage (ICH), subarachnoid hemorrhage (SAH), and other strokes. Moreover, the CBF and FIM are also influenced by a lot of factors such as age, gender, hematocrit, neurological deficits and contra-lateral lesions. Accordingly we focused on supra-tentorial unilateral stroke with CI and ICH to investigate the territory that strongly affected FIM.

There are apparent functional differences between the right brain and the left brain. The right hemisphere is important to space perception, and the left hemisphere is important to logical recognition including language. In this paper, we investigate the brain area, which is essential to obtain the better outcome in stroke rehabilitation, by means of the FIM and the CBF measurements.

METHOD

Subjects

One hundred ninety five first or relapsed stroke patients were admitted to our rehabilitation ward through our acute stroke unit or other acute stroke facilities during the period from March 2007 to November 2010. The medical conditions to be admitted to our rehabilitation ward were: (1) It is less than 2 months after the onset of stroke or its related surgery, (2). Effects of rehabilitation are expected in patients with hemiplegia, aphasia, ataxia, or mental disorders, etc. due to stroke. (3) There are no remarkable difficulties in staying at rehabilitation wards. (4). Patients planning to discharge to home are given priority. Comprehensive in-patient rehabilitation was given 5 days a week by our rehabilitation team, composed of 5 medical doctors, 6 physical therapists, 4 occupational therapists, 3 speech therapists, 3 social workers, and 15 rehabilitation nurses.

The inclusion criteria of this study were also the same as we reported as: (1) patients 25 days or above after onset, avoiding the acute and sub-acute stroke patients; (2) no major complications and no re-stroke during the stay in the rehabilitation ward; (3) confidence values of Xenon CT less than 1.4, which derived from an average + 2 SD (standard deviation) value obtained initial 120 cases of Xenon CT examinations; (4) hematocrit values more than 31%, which were led from the minimum value of female control [4]. In addition, we excluded the patients with SAH, infratentorial stroke and bilateral hemispheric stroke with old lesions more than 1cm in size in the non-newly affected lesion side.

Written or verbal informed consent was obtained from all patients prior to participation, and the ethics committee of our hospital approved this study.

CBF Measurement

CBF images were obtained using a Toshiba Asteion or a Toshiba Asterion multi-detector CT (MDCT) with 8 mm thickness $\times$ 4 slices and an Anzai AZ-726 stable xenon gas rebreathing system just before moving from the acute stroke unit to the rehabilitation ward. Serial 9 images were processed with AZ-7000W98 at the level of upper midbrain, thalamus, and lower and upper lateral ventricles, parallel to the orbito-meatal line. Regions of interest (ROIs) were drawn on frontal cortex, parieto-temporal cortex, occipital cortex, putamen, thalamus and whole hemispheric area at the level of thalamus for mCBF. The cortical rCBF values were averaged with upper 3 slices as shown in our previous report. To reduce motion artifacts, patients were secured with a vacuum lock head holder and earplugs we designed [4].

Functional Independence Measure

Functional outcome was assessed using FIM, which contains 13 subcategories of motor function and 5 subcategories of cognitive function. The FIM measures self-care (6 items), sphincter control (2 items), transfers (3 items), locomotion (2 items), communication (2 items), and social cognition (3 items). Each item, where 1 represents total dependence and 7

indicates complete independence, is scored from 1 to 7 based on level of independence. Possible total scores range from 18 to 126, with higher scores indicating more independence.

Statistical Analysis

Statistical analysis of the data was performed using EXCEL for Windows. Descriptive statistics were used to present the nature of all variables collected, including age, gender (male), CBF values, hematocrit values, FIM scores at discharge, onset to Xenon CT examination interval (OXI), length of chronic rehabilitation (LOCR), which length means the duration staying in the rehabilitation ward, national institutes of health stroke scale (NIHSS) at the time of Xenon CT examination, and lesion size, which is allocated 1, 2, and 3 in cases of maximum diameter of a dominant lesion less than 2 cm, 2 - 4 cm, and more than 4 cm respectively. The categorical factor of gender (male) was dummy-coded in the analysis: a positive factor is allocated 1 and a negative factor is allocated 0. Percentages, mean values, SD, the uni-variate analysis of variance, chi-test, and the analysis of regression coefficient were calculated where appropriate. Paired samples t-test was used to determine if there were any significant differences in right and left mCBF values. Multiple linear regression analysis in a stepwise manner was used to discriminate variables affecting FIM score at discharge. Spearman and Pearson correlation coefficients were used to examine the strength of relations between CBF values and FIM scores. The level of statistical significance was set as $p < 0.05$.

RESULT

A total of 130 patients, which consisted of 80 (62%) CIs and 50 (38%) ICHs were enrolled in the study. There were 78 men (60%) and 52 women (40%). The mean (SD, range) age was 67 years (13, 24-94 years). We excluded 12 patients with SAH, 24 patients with bilateral lesions and 29 patients with posterior-fossa lesions. The OXI was 38 days (10, 22-86 days) in total, 39 days (10, 22-57 days) in the CI group, and 38 days (11, 24-86 days) in the ICH group. Demographic and clinical characteristics of the patients were listed in Table 1.

Absolute average mCBF values of right and left were 25.1 and 28.4 ml/100g/min in the right CI group ($n=46$), 29.8 and 26.0 ml/100g/min in the left CI group ($n=34$), 24.7 and 30.7 ml/100g/min in the right ICH group ($n=29$), 33.0 and 27.9 ml /100g/min in the left ICH group ($n=21$), respectively. Mean CBF values on average of the lesion side decreased significantly with differences of 3.3 ml/100g/min in the right CI group, 3.8 ml/100g/min in the left CI group, 6.1 ml/100g/min in the right ICH group, and 5.1 ml /100g/min in the left ICH group, in comparison with the non-lesion side.

There were significant correlations of mCBF values between lesion sides (y) and non-lesion sides (x) in the right CI group ($R^2=0.560$, $y = 0.827x + 1.62$), the left CI group ($R^2=0.420$, $y = 0.853x + 0.569$), the right ICH group ($R^2=0.595$, $y = 0.783x + 0.618$), and the left ICH group ($R^2=0.724$, $y = 0.725x + 3.96$). The analysis of regression coefficient showed that there were no significant differences among 4 groups, which analysis means that the mCBF of the non-lesion side varies according to change of the mCBF of the lesion side.

Table 1. Demographic and clinical characteristics of patients (n=130).

Characteristics		infarction		hemorrhage		P
		right	left	right	left	
	n	46	34	29	21	
age	mean, sd	73, 11	69, 12	59, 10	61, 14	<0.001
gender	male, female	28, 18	20, 14	20, 9	10, 11	0.504, ns*
size(1-3)	mean, sd	1.8, 0.9	2.1, 0.9	2.6, 0.6	2.5, 0.7	<0.001
NIHSS	mean, sd	6.7, 4.2	6.1, 4.3	6.1, 3.9	7.7, 5.4	0.599, ns
LOCR	mean, sd	72, 24	65, 24	76, 32	86, 26	0.034
OXI	mean, sd	40, 10	38, 10	38, 12	38, 10	0.788, ns
hematocrit	mean, sd	39, 5	40, 4	41, 4	40, 4	0.708, ns
total FIM	mean, sd	89, 27	102, 25	108, 18	105, 16	0.0041

AVOVA, *: Chi-test, sd: standard deviation

size 1: <2cm, size 2: 2cm=<, 4cm=<, size 3: 4cm<

LOCR: length of chronic rehabilitation, OXI: onset to Xenon CT examination interval

Figure 1 shows the relation between mCBF values in the right and left sides, and FIM total scores at discharge. The mCBF values were significantly related with the FIM total scores in each group. The white triangle shows right side lesion group, and the black round shows left side lesion group. Although the correlation coefficient of the right mCBF ($r=0.375$, $p<0.001$, $n=130$) was slightly higher than that of the left mCBF ($r=0.333$, $p<0.001$, $n=130$), there is no significant difference of the correlation coefficients between them.

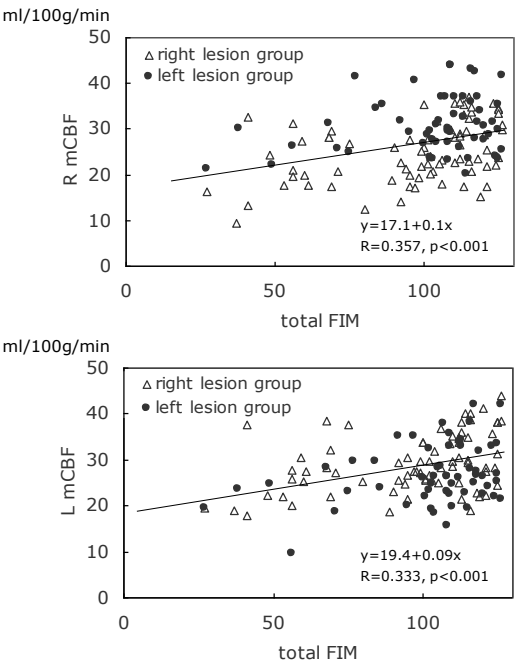


Figure 1. The relation between mCBF values in the right and left sides, and FIM total scores at discharge.

Multivariate analyses were performed for the purpose of identifying the factors that influenced FIM scores at discharge. Although, the analysis was carried out for all potential predictive variables and the right and left mCBF values, correlation matrix among predictive variables revealed that moderate multi-co-linearity was observed in relations between hematocrit and gender ($r=0.496$), size and NIHSS ($r=-0.405$), and right and left mCBF values ($r=0.567$).

Consequently, the factors of hematocrit, size and left mCBF values were deleted from the analysis. Stepwise backward elimination revealed that age, gender (male), NIHSS and right mCBF were significant factors ($R=0.788$, $R^2=0.621$, $P<0.001$) for the FIM total scores (Table 2). The analysis mentioned above led a formula for predicting FIM scores at discharge: Predicted FIM total score = $149-0.87A+6.1G-2.7N+0.79C$, A: age, G: gender male=1, female=0, N: NIHSS at the time of Xenon CT examination, C: right mCBF (Table 2).

Table 2. Multiple regression analysis for the FIM total scores.

stepwise-backward elimination					
Variables	Coefficients (β)	SE	95% CI	t	P value
Intercept	149.2	11.4	126.6 - 171.7	13.08	<0.001
Age	-0.87	0.11	-1.08 - -0.65	-7.96	<0.001
Gender (male)	6.15	2.94	0.32 - 12.0	2.09	0.039
right-mCBF	0.79	0.2	0.39 - 1.19	3.91	<0.001
NIHSS	-2.71	0.32	-3.34 - -2.08	-8.47	<0.001

$R=0.788$, $R^2=0.621$, $n=130$, $P<0.001$

SE: standard error, CI: confidence interval

Table 3. Relationship between mCBF and total FIM in supratentorial cerebral infarction.

	right mCBF		left mCBF	
	rs	r	rs	r
right cerebral infarction	0.508	0.47	0.465	0.433
n=46	$p<0.001$	$p<0.001$	$p<0.002$	$p<0.01$
left cerebral infarction	0.18	0.302	0.251	0.344
n=34	$p=0.310$	$p=0.0826$	$p=0.153$	$p=0.0464$

Rs: Spearman's rank correlation coefficient

R: Pearson's correlation coefficient

Spearman's rank correlation analysis revealed that the FIM total score of right CI was significantly correlated to both the right ($rs=0.508$, $p<0.001$) and the left ($rs=0.465$, $p<0.002$) mCBF, however, that of left CI was not correlated to the right mCBF ($rs=0.18$, ns) nor the left mCBF ($rs=0.251$, ns). Because the FIM was treated as a quantitative variable clinically, we also performed Pearson's correlation analysis in the same groups. There were significant correlations between FIM total scores and both the right ($r=0.47$, $p<0.001$) and the left ($r=0.433$, $p<0.001$) mCBF values of the right CI group and between FIM total scores and the

left mCBF values ($r=0.344$, $p=0.0464$) of the left CI group (Table 3). The right mCBF of left CI group did not show the significance ($r=0.302$, ns).

According to the subgroup analysis of the territories about cortical and perforating arteries, only the group of right CI with lesions in cortical artery territories showed significant correlation coefficients between FIM total scores and both the right ($r=0.656$, $p<0.001$) and the left ($r=0.751$, $p<0.001$) mCBF values. Nevertheless, there were no significant relations in the all groups with lesions in perforating artery territories and in the left CI group with lesions in cortical artery territories, except a slight tendency ($r=0.536$, $p<0.059$) shown in the right mCBF of the left CI group with lesions in perforating artery territories (Table 4).

We also studied the correlation between rCBF values and FIM sub-scores in CI. The FIM motor scores were significantly correlated with the rCBF of right putamen ($r=0.428$, $p<0.05$), right thalamus ($r=0.466$, $p<0.05$), right frontal cortex ($r=0.354$, $p<0.05$), right parieto-temporal cortex ($r=0.414$, $p<0.05$) right occipital cortex ($r=0.415$, $p<0.05$), left putamen ($r=0.458$, $p<0.05$), left parieto-temporal cortex ($r=0.362$, $p<0.05$) and left occipital cortex ($r=0.437$, $p<0.05$) in the right CI group, and the rCBF of right thalamus ($r=0.379$, $p<0.05$) and right frontal cortex ($r=0.345$, $p<0.05$) in the left CI group.

Table 4. Correlation coefficients between total FIM scores and mCBF depending on the territories of cerebral infarction.

	territories	n	right mCBF		left mCBF	
			r	p	r	p
right cerebral infarction	cortical artery	23	0.656	<0.001	0.751	<0.001
	perforating artery	23	0.291	ns	0.1	ns
left cerebral infarction	cortical artery	21	0.273	ns	0.347	ns
	perforating artery	13	0.536	0.059	0.235	ns

On the other hand, FIM cognitive scores were significantly correlated with the rCBF of right putamen ($r=0.368$, $p<0.05$), right thalamus ($r=0.342$, $p<0.05$) and left putamen ($r=0.385$, $p<0.05$) in right CI group, and the rCBF of right putamen ($r=0.343$, $p<0.05$) and left putamen ($r=0.477$, $p<0.05$), left thalamus ($r=0.421$, $p<0.05$) and left frontal cortex ($r=0.520$, $p<0.05$) in the left CI group. As a common result of right and left CI groups, the rCBF values of right frontal cortex and the rCBF values of right thalamus were significantly correlated to FIM motor scores, and the rCBF values of right and left putamen were significantly correlated to FIM cognitive scores (Table 5).

As for ICH, we analyzed the relation between mCBF and FIM total scores according to location of the hematoma; however, the ICH did not show the same results as CI. There were no significant relations in the group of right putaminal hemorrhage ($n=16$, right mCBF: $r=0.260$, ns, left mCBF: $r=0.214$, ns), right thalamic hemorrhage ($n=9$, right mCBF: $r=0.625$, $P=0.072$, left mCBF: $r=0.388$, ns), left putaminal hemorrhage ($n=6$, right mCBF: $r=0.262$, ns, left mCBF: $r=0.190$, ns), and left thalamic hemorrhage ($n=11$, right mCBF: $r=0.201$, left mCBF: $r=0.230$, ns).

Table 5. Correlation coefficients between regional CBF and FIM sub-scores in cerebral infarction.

diagnosis	side	FIM	putamen	thalamus	frontal	parieto-temporal	occipital
right cerebral infarction	right	motor	0.428***	0.466***	0.354*	0.414***	0.415***
n = 46		cognitive	0.368*	0.342*	0.215	0.25	0.275
	left	motor	0.458***	0.271	0.208	0.362*	0.437***
		cognitive	0.385**	0.182	0.202	0.261	0.399**
left cerebral infarction	right	motor	0.127	0.379*	0.345*	0.025	0.135
n = 34		cognitive	0.343*	0.283	0.231	0.002	0.139
	left	motor	0.206	0.206	0.305	0.165	0.257
		cognitive	0.477***	0.421*	0.52**	0.332	0.214

*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.005$

DISCUSSION

The main result of this study was that the mCBF of CI with right hemispheric lesions showed significantly correlated to FIM scores at hospital discharge but not the CI with left hemispheric lesions and the ICH. In details we described 7 results below: 1 Mean CBF of no lesion side decreased in proportion to mCBF of lesion side. 2. FIM total scores exhibited weak but significant relations to right mCBF and left mCBF in the whole group. 3. The factors of age, gender and NIHSS also influenced the FIM total scores. 4. The FIM total scores significantly correlated to right and left mCBF in the CI with right hemispheric lesions, but not in the CI with left hemispheric lesions. 5. The common structures of both right and left CI groups, which showed significant relation between rCBF values and FIM sub-scores, were right thalamus and right frontal cortex in FIM motor scores and bilateral putamen in FIM cognitive scores. 6. The CI in the cortical artery territories showed a significant relation between right and left mCBF, and FIM total scores, however not in the perforating artery territories 7. The ICH group did not showed significant correlations between mCBF and FIM total scores.

Since the interval between the time of CBF measurements and hospital discharge were about 2 months and xenon CT was performed in resting condition, the CBF values measured do not always reflect the actual brain activity during rehabilitation exercises. We consider the CBF value as the potential ability to carry out the rehabilitation program during stay in the ward. Besides, we should understand the characteristics of ROI in CBF studies. When the CBF is indicated at a low value, there are two possibilities. One is a case with the brain area in low activity. The other is a case with the ROI including no flow area such as sulcus, cistern, ventricle, cyst and necrotic lesions. Basically some gaps in ROIs were not avoidable, even if the area damaged on structural imaging was excluded in CBF analysis. Nevertheless, the rCBF values measured in ROIs seems not to be so different from actual rCBF values, because the rCBF values adjacent to lesions were always reducing.

The FIM is an ordinal scale, and then we used both Spearman rank correlation coefficient and Pearson correlation coefficient in order to ascertain the statistical relation between FIM

scores and CBF values. It is clinically used as a quantitative scores and the increase of scores reduces nursing cares. Accordingly, the FIM score is an effective parameter to evaluate the rehabilitation outcome. As Tur BS et al. reported that significant correlations were found between Brunnstrom's motor recovery stage and FIM scores both on admission and at discharge [5], the initial FIM score on admission of rehabilitation ward might be also useful to anticipate the stroke outcome.

Diaschisis is a kind of transneural suppression, and it reduces activity in connecting brain area remote from the lesion site due to decrease of the excitatory impulse from an injured region. Many authors showed the CBF decreases in a variety of diaschisis in the acute stage [6]. In the chronic stage, as we showed, mCBF values of the non-lesion side significantly decreased according to the decrease of CBF values in the lesion side. This result therefore might be explained with a transcallosal diaschisis.

Indifferent to lesion sides in our series, there were weak but significant relations between FIM total scores and both right and left mCBF values. One of the causes is speculated that the each hemisphere has own function influencing the ADL. There is an example of the contribution of the right and left hemispheres to recovery from aphasia in SPECT study [7]. They discussed the importance of both non-dominant hemisphere and language dominant hemisphere in efficient recovery of linguistic abilities, though their study was not for ADL or for chronic stroke.

Multivariate analyses revealed that age, gender, NIHSS, and CBF values were significant factors influencing FIM total scores. In general the CBF decreases with healthy aging, however it is controversial matter. Meltzer CC et al. reported that CBF may not decline with age in healthy individuals and that failure to correct for the dilution effect of age-related cerebral atrophy may confound interpretation of previous PET studies that have shown aging reductions in physiologic measurements [8]. Average CBF values of female are higher than those of male [9]. This means that a female patient shows higher CBF values than a male patient when they had the same ADL level. The factors of aging, female and NIHSS show negative influence to the outcome and the factors of CBF increase and male lead better outcome and thereby, we need to take these factors into consideration to estimate the CBF values.

Rupright J et al. showed a strong correlation between CBF and improvement of FIM scores, especially in the right brain of ischemic stroke [10]. Goto A et al. reported that the locomotion outcome was poorer in patients with a right hemispheric CI than a left hemispheric CI for the patients with middle cerebral artery infarction [11]. Shulman GL et al. reported that right hemisphere dominance during stimulus-driven shifts of spatial attention and target detection reflected asymmetries in cortical regions in the functional MRI study [12]. We also demonstrated significant relations between FIM total scores and bilateral mCBF values in the right CI group, especially with lesions in the cortical artery. The left brain deals with verbal, logical and analytic abilities, and the right brain mainly deals with spatial recognition, which function is essential to move a body more stably. Accordingly, these results seem to explain that the right brain has more influential role in better rehabilitation outcome than the left brain.

The human brain looks symmetrical, but there are several reports, which explain asymmetry. MRI revealed the asymmetries of the human brain in the review of molecular approaches [13]. Left-right asymmetry of the hippocampal synapses in primates was also reported [14].

In the detailed analysis common to both right and left CI groups, rCBF values of right frontal cortex and right thalamus showed significant relations to FIM motor scores, and rCBF values of bilateral putamen showed significant relations to FIM cognitive scores. The FIM motor score measures physical independence and corresponds to the Barthel index, and assesses eating, grooming, bathing/showering, dressing upper body, dressing lower body and toileting as the items of self care, bladder management and bowel management as the items of sphincter control, bed/chair/wheelchair, toilet, bathtub/shower as the items of morbidity, and walking/ wheelchair and stairs as the items of locomotion. The spatial recognition is indispensable to move our bodies correctly. It is reported that the right putamen, caudate nucleus, thalamus and superior temporal gyrus form a coherent corticosubcortical anatomical network in the genesis of spatial neglect in humans [15]. Therefore, our results about the relations to FIM motor scores of the right frontal cortex and the right thalamus agreed with the idea that spatial recognition is dominant in the right hemisphere. The FIM cognitive score assesses both comprehension and expression as the items of communication, and assesses social interaction, problem resolving and memory as the items of social cognition. The result that the bilateral putamen related to the FIM cognitive score implies that both left brain, which is dominant in language, and right brain are influential to communication and social cognition.

The main function of the putamen is regarded to regulate movements, and it is closely connected with substantia nigra that employs dopamine to perform its function. Cerebral cortex, basal ganglia including putamen, and thalamus have also close connections, unilaterally and bilaterally. The sensory information of the body is transmitted to parietal cortex and other cortices through thalamic radiation. In addition, the frontal lobe is a major part of dealing with activities. Degenerative disorders, such as Parkinson's disease, cause a shortage of dopamine. There was an imaging study of Alzheimer's disease, which showed the decrease in putaminal volume correlated linearly with impaired global cognitive performance [16]. Another study showed that stroke patients with basal ganglia lesions were impaired on the rule-based task, not in the information-integration task [17]. These studies suggest that the putamen takes part in the recognition activity. On the other hand, there was a report that atrophy of the putamen in dementia with Lewy bodies is a parkinsonian feature, not correlating with dementia in the volumetric MRI study [18].

The relation between FIM and CBF in the group of ICH was different from that in the group of CI. The ICH group did not show a significant correlation between FIM total scores and mCBF values. Katrak PH et al. showed that patients with ICH achieved significantly greater gains in function than patients with CI after rehabilitation regardless of the severity of disability on admission [19]. Schepers VPM et al. also reported that the time window for recovery was more restricted for patients with ICH; an increase in ADL independence was found until 10 weeks post-stroke in patients with ICH, whereas patients with CI showed recovery until 26 weeks post-stroke [20]. We speculate that intracerebral hematoma compresses adjacent normal brain tissue and causes a decrease of CBF in the adjacent tissue until early in the chronic stage, and then, a marked reduction of hematoma volume leads the CBF recovery. Accordingly, CBF values of patients with ICH cannot always correlate with stroke outcomes after rehabilitation, whereas the metabolism of cerebral tissue of CI is more directly affected by the hypo-perfusion for a long time. In our series, the OXI of ICH group is not different from that of CI group. However, the CBF of ICH group might be related to FIM, if the CBF of ICH group was measured after the disappearance of hematoma.

The human brain has a dominant hemisphere, but little is known about the reason why right and left differences develop. Some animals can sense the Earth's magnetic field and use it as a cue for guiding movements over both long and short distances [21]. And it is considered to be a kind of spatial recognition. All the animals are influenced by environment such as gravity, rotation and magnetism of the Earth. Consequently, these natural phenomena might have influenced the development of dominant hemisphere in the human brain.

CONCLUSION

This study revealed that the right and left hemispheres influence mutually, and there is a significant relation between the CBF values and its outcome, especially in case of right brain lesion. In addition, this study implies that the putamen is related to cognitive function.

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Chapter 44

THE REHABILITATION FUNCTION OF MOTOR IMAGERY AFTER PERIPHERAL INJURY OR CENTRAL STROKE

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ABSTRACT

This paper investigates the rationale for integrating Motor Imagery (MI, the representation of an action without any overt execution) into the classical course of physical therapy to improve motor recovery. This therapeutic approach is based on comparable structural and functional properties shared by actual execution and MI. The main damages at the central level are cerebral stroke and spinal cord injury (SCI). Hemiplegia or hemiparesis following stroke could drastically alter the contralateral motor function. In addition, somatosensory information and language may also be affected, depending on the extent of brain damage. Conversely, cognitive functions such as memories, attention, problem solving (e.g., mental arithmetic, mental travel) or MI remain generally preserved, albeit with some exceptions we will briefly consider. Therefore, it should be possible to start therapy based on mental practice early on during the rehabilitation program. Indeed, after the acute phase, there is a period during which usual rehabilitation is difficult and harmful due to the general state of the patient. This is a good time-period for starting rehabilitation with MI. SCI affect lower limbs' motor function or even the four limbs, depending on the height of the spinal lesion. In the first case, locomotion is most often removed whereas in the second, upper limb functions, e.g., pointing, grasping and manipulating objects are more or less altered. We will review the main papers focusing on SCI, at least paraplegia with specific focus on posture and locomotion, and tetraplegia with specific focus on grasping. Then, we will provide several data related to the use of MI in the rehabilitation of motor function after

peripheral injuries of different natures, e.g., joint sprain, muscle tear or bone fracture. Finally, we will describe how MI could also be considered a reliable preventive technique for preserving postural functions and locomotion especially in a vulnerable population, the elderly. We will conclude by underlying the curative and the preventive function of MI training, while considering the importance of the practice guidelines ensuring effective MI and the thorough assessment of patients' ability to form and manipulate mental images.

INTRODUCTION: MOTOR IMAGERY AND MOTOR LEARNING

Pioneering studies provided evidenced that motor imagery (MI) training had the potential to improve motor learning efficiency, when combined with actual execution. Feltz and Landers (1983) were among the first who extensively studied how different forms of mental practice, including MI, could enhance motor skill learning and sport performance. This meta-analysis showed that performing MI was not as efficient as actual execution but provided better performance than that observed in a control (i.e., no training) condition. In their revised meta-analysis (Feltz et al., 1988), the same authors confirmed these previous results. Driskell et al. (1994) further reiterated that mental practice has a positive and significant effect on performance. Its effectiveness was nevertheless moderated by the type of task, the retention interval between practice and performance, and the length or duration of the mental practice intervention, as it is observed during actual practice. In the present paper, we focus on motor function rehabilitation, a particular domain of MI intervention. We will first briefly describe the other potential domains in which MI could operate and then give the rationale for using MI under such conditions. However, several concerns related to MI use in the context of rehabilitation will be described in details. Finally, we will present several experiments in which MI brought insightful and promising results for clinical applications. Practically, we will consider central nervous system injuries (i.e., stroke and SCI) and separate these from several examples of peripheral lesions.

RELATIONSHIPS BETWEEN MI AND ACTUAL EXECUTION

Human-being has the ability to produce an internal representation of a specific motor action without any overt motor output. It is assumed that this process could help motor learning because mental practice based on MI requires the participants to have the necessary declarative knowledge related to the different features of the motor task. According to Jackson et al. (2001), the rehearsing of the task with MI can also give access to the non-conscious processes involved in learning the skilled behavior, as with physical practice. In groups of athletes or musicians who extensively mentally rehearse specific skills, several MI types could be used. Visual clues are the main material for mental practice in activities with spatial aim (tennis or ski, for example), whereas kinesthetic information is probably more useful in sports with bodily-centered techniques. Finally, musicians could primarily use auditory MI or more simply the pace and the rhythm at which a piece of music is carried out. Another remarkable domain in which MI is now considered a useful tool is for surgical skills teaching (Hall, 2002). Imagery practice provides a mechanism for the explicit learning of

surgical skills. Surgeons could train themselves by applying a set of declarative instructions mentally, i.e., associating declarative knowledge into procedural abilities (Arora et al., 2011a). They could also process stress related to surgery using MI (Arora et al., 2011b). In this latter study, decreased stress was attested through the STAI test (State-Trait Inventory test from Spielberger et al., 1970) associated with a set of physiological measures, including mean and maximal heart rate as well as cortisol level. During MI, an increase in muscle tension has often been described (Lotze and Halsband, 2006). Activation of the muscles involved in mental action could increase up to visible movements (e.g., fingers in violin players or hand movements in skiers). Considering such associated muscle activity implies that this mental practice should no longer be called MI remains to be discussed. However, it seems obvious that internally driven images would benefit from kinesthetic information and would consequently better activate the infra-conscious processes that are associated with motor task execution (Jackson et al., 2001). According to Jeannerod (1994), the neural commands for muscular contractions are probably blocked at some level of the motor system, thus preventing to send the full command within the motor pathways and inhibiting motor execution. However, a slight electromyographic (EMG) activity has often been recorded during the imagination of movement. Jeannerod (1994) early postulated that incomplete inhibition of the motor command would provide a valid explanation to account for this muscle activity. Recent data support this hypothesis. Guillot et al. (2007) provided evidence of specific changes in EMG activity according to each type of contraction, i.e., isometric, concentric, or eccentric. Interestingly, the changes in the EMG pattern mirrored those observed during physical movement, thus supporting the hypothesis of a selective effect of MI at the level of muscular activity. For example, imagining an eccentric contraction elicited a significant weaker EMG activity than during all other conditions. This result is consistent with a first validation of the hypothesis of incomplete inhibition of the motor command.

With reference to the data that we just described, MI seems functionally close to actual movement execution. As many studies previously showed that the neural networks controlling movement and the mental representation of the same movement overlap, MI could be seen as a part of the motor system (Lotze and Halsband, 2006; Hanakawa et al., 2003; Dechent et al., 2004; Porro et al., 1996; Lacourse et al., 2005). Stephan and Frackowiak (1996) compared MI with motor preparation and motor execution using positron emission tomography and described their respective functional anatomy. They also monitored EMG, Hoffmann's-reflexes and transcranial magnetic stimulation to delineate their electrophysiological characteristics. Both studies demonstrated that MI shares some characteristics with motor preparation and other additional characteristics with motor execution. They concluded that MI might be seen as a specific form of motor behavior, similar but distinct from both motor preparation and execution. Combining the mutual features of actual execution and MI, this particular type of mental rehearsal may be one potential key to enhance motor learning. Thus, as described before, MI can be applied to a wide range of populations, e.g., to train athletes, musicians or surgeons, but could also be associated with traditional rehabilitation programs of motor functions. In several recent reviews, the authors underlined that specific MI protocol, which could be – or not – associated with movement observation sessions, could improve motor recovery in the affected limbs in people living with the chronic effects of stroke (e.g., McEwen et al., 2009; Mulder, 2007).

CONDITIONS REQUIRED BY MI TRAINING DURING MOTOR REHABILITATION

First, we must investigate whether the consequences of stroke still enables patients to mentally perform some of the movements they are no longer able to actually execute (Johnson, 2000). Many patients with hemiparesis or hemiplegia preserve the ability to represent themselves grasping an object with their impaired limb. This is a strong argument for programming MI sessions, if not during the acute phase, at least within the chronic period with the aim to facilitate functional reorganization. However, clinicians should control the exact extent of the lesion with accurate cerebral neuroimaging techniques, to provide a diagnosis of what could be exactly mentally performed by the patients. This ability may be impaired in case of right posterior parietal or left frontal lesions, known as being integrated in a network of interconnected areas distributed throughout parietal and frontal regions. One of the most important skills to recover after stroke is acting within the haptic space to make grasping available. Grasping an object remains nevertheless different from using this object. Tool use engages a left hemispheric network including frontal, temporal and parietal regions and patients with left brain lesions exhibit apraxia, i.e., deficits when demonstrating how a single tool should be used. This is an important issue as MI should be based on goal-directed movement to make this exercise meaningful for patients. Tool impairment reveals little overlap of cerebral regions with those controlling grasping. These two motor functions remain nevertheless linked together as choosing a functional grasping movement depends on the goal of the task and the online information about the object's structure and orientation (Randerath et al., 2010). Thus, patients exhibiting such cerebral lesions are probably not the best candidates for being involved in a MI rehabilitation program. According to Simmons et al. (2008), almost 40% of the patients are unable to perform MI after stroke. Although inter-individual differences in MI abilities were high, due to the various features and consequences of stroke, this observation would probably prevent from programming MI sessions for such patients or, at least, this is an indication for postponing mental work after some potential recovery due to cerebral plasticity (Cramer, 2008; Rossini and Dal Forno, 2004). Despite this, Zimmerman-Schlatter et al. (2008) reviewed studies focusing on the efficacy of MI in post-stroke rehabilitation, and concluded that MI provides additional benefits to conventional physiotherapy. Another concern is related to the involvement of primary motor cortex in MI as it is at the interface between the operations of movement preparation and execution (Dechent et al., 2004). After stroke, the movements opposite to the stroke side, whenever possible, activate the contralateral primary motor cortex, as also observed in healthy participants. Simultaneously, the same movement was shown to activate the ipsilateral motor area, i.e., that of the unaffected hemisphere (Ward et al., 2003; Calautti et al., 2007). The extent to which this could impair the MI function remains rather unknown. As underlined by Sharma et al. (2009), the motor system, including the ipsilesional primary motor cortex, is activated by MI in well-recovered patients after subcortical stroke. There remains, however, a suspicion of cerebral disorganization with reference to residual motor impairment and within this context, the effect of MI training may potentially be weakened. The main factors that determine the use of MI and the quality of the results to be expected were recently described by Dijkerman et al. (2010). Among them, the authors outlined the severity of motor impairment, the time since onset of motor impairment, the cognitive impairments and, of

course, the ability to form mental images. We nevertheless keep the possibility to evaluate the quality of MI by using other indirect indicators, e.g., behavioral outcomes. The measure of behavior is based on the functional equivalence between the actual execution of the movement and its mental representation. The pace at which MI is performed closely resembled that of actual execution. It is easy to measure the time of a mental representation. The principle of isochrony is ideally applicable to motor sequences lasting from 10 to 20 seconds. Knowing the actual movement duration, the comparison with the time needed to mentally perform the same sequence provides information about the participant's ability to preserve the temporal characteristics of the movement. Above and below these boundaries, time is distorted (Guillot and Collet, 2005). Forming a vivid image of a very short movement requires time and, therefore, the actual duration is frequently overestimated. Conversely, a very long motor sequence is likely to be shortened when rehearsed, especially if it is rhythmic or repetitive, e.g., walking along long distances. Keeping these particular cases in mind, measuring the time needed to mentally perform a movement is one of the easiest means for the evaluation of MI quality (Malouin et al., 2008). Due to general motor impairment after stroke, however, it may be assumed that the principle of isochrony will be changed in stroke patients. Wu et al. (2010) clearly showed that participants required significantly more time to physically practice than to mentally perform tasks, on the one hand. On the other, stroke also drastically affected MI as a significant greater amount of time for mental practice of the more-affected arm than for the less-affected arm was also observed. Mental chronometry could thus be used to assess the severity of stroke and to evaluate how patients improve movement timing along the rehabilitation process.

FEEDBACK INFORMATION DURING ACTUAL EXECUTION, MI AND AFTER DEAFFERENTATION / DEEFFERENTATION

An important concern is related to the somesthetic peripheral information which is not longer available in patients with stroke. Such information is however needed to perform a movement adequately and, consequently, an accurate mental representation. The mental representation of an action is probably one of the first steps before its actual execution. At least, the individual should represent the main goal of the movement and the own consequences of the action (in terms of result or motor performance). This is associated with some expected sensorial perception which is recalled from the procedural long term memory and serves as reference for the forthcoming execution. The well known contribution from Schmidt (1975) was updated in 2003 and stated that during the preparation phase, the individual could predict the expected feedback, either visual or somesthetic, that the actual movement should provide (Schmidt, 2003). The movement is considered well-done when the expected information matches the actual outcome. In contrast, when the actual feedback mismatch the expected feedback, the individual could consider, with the help of his (her) trainer or teacher, that the actual execution is better or worse than the previous one. In the first case, he (she) should memorize the new execution recognized as being more efficient. In the second, he (she) might consider that the actual execution should not be memorized as a new model of movement and should, at least, just be seen as an experience that should not be repeated in forthcoming trials. In the particular case of MI, sensorial information related to

movement execution can be recalled without any overt execution according to a ‘top-down’ operation. Such information, corresponding to the expected feedback, could however not be compared to actual execution. This is the basis of MI training. While the close relationship between motor processes and imagery is often underlined, stroke patients may not benefit from sensorial feedback. They could just rely on central information that cannot be updated through actual execution. The main role of MI could thus be reduced to the maintenance of motor information in the long term memory across time. If SCI patients are believed to allocate additional cognitive resources to perform MI, they were nevertheless shown to preserve the integrity of motor programs at the central level. Accordingly, Hotz-Boendermaker et al. (2008) provided evidence that not only the MI of foot movement activated the premotor and motor cortex, but also a real attempt to execute the same movement. The authors thus considered that central programs were preserved despite long-term deafferentation / deefferentation. However, Lacourse et al. (1999) previously showed that cortical motor processes were altered by the absence of kinesthetic feedback during attempted movement of a deafferented limb as well as during MI. Whether central programs are preserved seem to be attested by experimental data but another process could work simultaneously to reorganize central networks. The extent to which this reorganization could occur is not known in greater details as, at our own knowledge, only two papers specifically addressed this issue (Jackson et al., 2003; Sacco et al., 2006). On the other hand, somesthetic perception, although altered, is not entirely removed. At least, stroke patients could probably just perceive weakened information remaining from body segments during passive exercise of limbs manipulation by the physiotherapist. In addition, they could also use visual information for controlling movement execution that should usually be controlled through proprioceptive and tactile information. In this latter case, the vision system serves a proprioceptive function. Somesthetic cues are not the only source of available information during actual execution and several papers demonstrated that autonomic nervous system activity is preserved during MI (Decety et al., 1993; Roure et al., 1998; Oishi et al., 2000; Collet and Guillot, 2010). The question of whether autonomic responses during MI could be used by patients to improve motor execution could thus be asked. Heart rate and electrodermal responses are observed as early as the participant is engaged in the mental representation of an action. However, these autonomic responses could not be consciously perceived and we may hypothesize that providing a feedback of what responses the mental rehearsal has elicited might help the patients during the rehabilitation process. Patients could be advised to focus themselves on important features of the movement they should mentally rehearse, and could then read the information displayed online by the physiological dataset. The association between a particular pattern of autonomic responses and the vividness of MI could thus be learnt by the patient, to serve as reference for effective MI. This procedure could be an opportunity to address the removal of sensorial feedback after stroke but also to monitor the quality of MI products in real-time.

Despite the limitations we mentioned about the consequences of central injury on MI ability (possible MI impairments and difficulty to integrate sensorial feedback while being paralyzed), the reference to MI intrinsic properties promotes that MI is a reliable candidate to be integrated into rehabilitation programs. We should now investigate the main pathological fields which are eligible for mental rehearsal.

MI TREATMENT IN PATIENTS WITH STROKE

Taken as a whole, all injuries keeping the patients motionless or require them to rest during the post-surgery period should benefit from mental training. We already described stroke as one of the main injuries which is likely to be treated with MI in association with traditional rehabilitation. Many papers have now demonstrated the beneficial role of MI in motor rehabilitation after stroke. Dickstein et al. (2004) integrated MI sessions for gait rehabilitation in post-stroke hemiparesis. A 69-year-old man with left hemiparesis completed gait practice for a 6-week intervention focused on gait and impairments of the affected lower limb (a 15-minute-MI session, 3days a week). Several kinematics parameters were improved, e.g., 23% increase in gait speed and 13% reduction in double-support time associated with an increase in range of motion of the knee. The same program was applied to 4 persons with chronic hemiparesis and resulted in similar improvements of locomotion and gait (Dunsky et al., 2006). The most recent study by the same team included 17 participants with hemiparesis caused by unilateral stroke. The authors notably found that the walking speed significantly increased by 40% after training (e.g., single-support time of the affected limb and double-support time decreased). Stride length also improved and gains were largely maintained at the 3-week follow-up test (Dunsky et al., 2008). Recovery from stroke also requires the reconstruction of the upper-arm functions and particularly of hand skills. Page et al. (2007) recorded significant clinical changes in the rehabilitation of the affected arm in patients with chronic stroke using a program incorporating mental practice. Based on the hypothesis that MI is more effective when combined with task-specific practice, the authors requested 10 chronic stroke patients to complete 30-minutes sessions of mental practice directly after usual therapy. The dependent variables included two specific tests focused on manual abilities and clinical evaluation of daily task activities. After intervention, participants exhibited substantial improvement in both tests and significant performance gains in daily tasks. Interestingly, fMRI data in the follow-up period revealed significant increases in activation to wrist flexion and extension of the affected hand in the premotor area and primary motor cortex ipsilateral and contralateral to the affected hand, as well as in the superior parietal cortex ipsilateral to the affected hand. The authors attributed these changes to cerebral plasticity elicited by the therapy program and this was probably one of the first study exhibiting changes in the cortical maps in response to MI practice since the contribution of Pascual-Leone et al. (1995). Iestwaart et al. (2011) obtained more mitigated results by investigating the potential therapeutic benefit of MI in stroke patients with persistent upper-limb weakness. The study analyzed the outcome of 3 groups of participants: i) 39 patients were involved in 4 weeks of mental rehearsal of upper limb movements during 45-min supervised sessions three times a week and structured independent sessions twice a week, ii) the second group included 31 patients who performed equally intensive non-motor mental rehearsal, and iii) 32 patients received normal care without additional training. No difference between the treatment groups was found on the Action Research Arm Test (Lyle, 1981) or on any of the secondary outcome measures. The authors concluded that mental practice with MI does not necessarily enhance motor recovery during the early post-stroke period, at least if actual practice is not associated with the mental work. In light of such data, it remains to be seen whether mental practice with MI is an effective rehabilitation technique, or at least determine what the prerequisites to ensure its effectiveness are. It also remains to analyze the

content of mental rehearsal sessions as several differences could easily be seen when comparing experimental designs. Most tasks involved mentally rehearsed movements of upper or lower limbs. However, tasks included either single movement involving a limited number of muscles (flexion or extension), non goal-directed movements, or even movements with a clear objective close to that of daily life. Intervention period also varied from 2 to 6 weeks, while the frequency of MI sessions ranged from multiple sessions per day, up to 3 times a week. The dependent variables clearly differed with regards to patients' characteristics. There was some evidence that additional MI intervention has positive effects on arm recovery function after stroke. However, as underlined by Braun et al. (2006), no definitive conclusion could be drawn except that further research is needed with particular attention to the definition of mental training content (especially the type of MI which is believed the more effective, i.e., visual and/or kinesthetic) and standardized measurement of outcome. Several studies provided evidence that mental rehearsal of movement can elicit effects that normally result from actual practice. One of the most positive results is that MI intervention may elicit plasticity in the brain (Pascual-Leone et al., 1995). Despite changes in brain activation, it may be hypothesized that these have not elicited changes at the behavioral level yet. Variations in brain activity might thus not directly promote changes at the behavioral level and additional time might be needed to observe behavioral transformation. It seems obvious that further investigations should determine the appropriate load and the relative ratio of both physical and MI practice. This issue was recently addressed in a review paper by Schuster et al. (2011) with a specific exploration of what is actually done in 5 domains of activity using MI including clinical applications. Finally, one of the neglected MI properties is its ability to deal with psychological problem solving, by improving self-confidence and managing stress. Because of the urgency to deal with the motor consequences of stroke, it is common that the question of patients' psychological state is ignored. Yet, it seems clear that persons with stroke are truly stressed by the alteration of their physical integrity. The more severe the injury and longer the recovery period of rehabilitation, the more prolonged and important the mood disturbance may be, with potential consequences on motivation to recover motor function, at least partially. After experiencing stroke, the patients could feel several negative emotions including isolation (restricted relationships with family and friends), frustration (awareness of both the alteration of physical abilities and the difficulty or inability to recover mobility), and mood disturbance (the patient may temporarily be depressed, or become upset by minor annoyances). MI may have psychological therapeutic effects related to stress management and relaxation. The possibility of starting imagery, even during the acute phase, by focusing the patients on positive and relaxing images could be a fruitful preparation for the subsequent physical rehabilitation. Two theoretical models of MI, originally designed for sport performance, integrated the emotional dimension, i.e., the PETTLEP model by Holmes and Collins (2001 – P for Physical, E for Environment, T for Task and Timing, L for learning, E for Emotion and P for Perspective) and the MIIMS model by Guillot and Collet (2008 – Motor Imagery Integrative Model in Sport). In the first model, the authors hypothesized that including realistic emotional scenarios in the imagery instructions is likely to make imagery much more evocative and closer to real-life situations. Therefore, this should lead to a more vivid imagery experience. The MIIMS describes 4 domains of intervention for MI. Among them, there is a specific field in which MI may have a psychological outcome by controlling stress, improving self-confidence and enhancing motivation. These are dimensions which should now have a rehabilitation function due to the

particular psychological state the patients should undergo. MI has thus the potential to act at both levels, i.e., that of motor recovery with rehabilitation training and that of the more extensive psychological status of patients (decreasing stress and increasing well-being).

MI TREATMENT IN PATIENTS WITH SPINAL CORD INJURY

Although brain and SCI are related to different causes, the main consequences result in the paralysis of limbs or, at least, in severe alteration of motor functions (e.g., gait, locomotion, grasping). Low SCI paralyzes the lower limbs (paraplegia) while injury of the spinal cord at the level of cerebral vertebra results in both lower and upper-limbs paralysis (quadri- or tetraplegia). Paraplegia is observed in case of SCI at the level of the second lumbar vertebra or below (thus with specific motor deficits). Tetraplegia also results in several levels of injury with different motor consequences. Trauma at the fifth/sixth cervical vertebra could lead to severe paralysis of the four limbs whereas at the sixth/seventh cervical vertebra, some upper-limbs functions could be preserved, e.g., extension of the forearm. At our own knowledge, the first study focusing on MI after SCI is from Decety and Boisson (1990). They took movement time as the main dependent variable and observed that movement times in the group of paraplegic and tetraplegic patients did not differ from those of healthy participants. The authors reported that the patients felt a sensation of subjective effort accompanying mental rehearsal and used this observation to claim that MI should be compatible with rehabilitation. At the neurophysiological level, there is no discussion about whether cognitive functions may be impaired by SCI. This was later confirmed by Hotz-Boendermaker et al. (2008) as patients with complete SCI could nevertheless perform the mental representation of a movement involving a paralyzed body segment but could also attempt to perform this movement. Both operations were shown to elicit cortical activation, thus giving some evident support to motor program preservation despite both deafferentation and deafferentation. Sabbah et al. (2002) confirmed that, even several years after SCI from the sixth thoracic to the first lumbar vertebra, the activation of lower limb cortical networks can be generated either by the attempt to move, the mental evocation of the action, or the visual feedback of passive somesthetic stimulation. Nevertheless, Lacourse et al. (1999) underlined that, due to the absence of sensory feedback from the deafferented limbs, some variability in central motor processing could occur. The assumption that compensatory information originated from visual information could overcome the lack of somesthetic clues has not yet been tested (see the above paragraph *"Feedback information from actual execution, MI and after deafferentation/deafferentation"*). From a pragmatic viewpoint, how MI might contribute to promote the motor rehabilitation of SCI patients? Only few studies addressed this issue, by testing whether MI practice can improve motor function. There are no extensive studies which would have covered a large number of patients but only several case studies or groups with a small number of patients who completed an individualized MI intervention. Grangeon et al. (2011, submitted) applied a 5-week MI training program to a male patient injured at the C6-C7 level. He exhibited high MI abilities that would have probably favored significant improvement of motor function. Indeed, he considerably improved speed and accuracy of a reaching and grasping sequence with his dominant arm. Despite the enhancement of intrinsic movement speed, the improvement of movement

smoothness was also a factor that highly contributed to decrease movement time. MI sessions were made of single movements representation such as arm flexion and extension further associated with movement needed for daily-life activities, e.g., extending the forearm towards a target to be reached and seized. The MI program was combined with classical sessions of physiotherapy and ergotherapy. The patient also significantly improved normative and validated tests, e.g., the Minnesota and Box and Blocks tests. Due to the weakness of muscles controlling digit flexion, the patient was guided to learn another coordination requiring a new plan for muscles cooperation. When extending the arm towards the target to be reached and grasped (e.g., a glass), the patient was instructed to keep his wrist flexed. As early as his hand came into contact with the glass, he was requested to extend his wrist, this movement being passively helped by hand contact on the glass associated with the weight of the upper-limb. The wrist extension automatically triggered fingers flexion, thus enabling to grasp the glass. Once the glass was held, the patient should maintain his wrist extended to ensure keeping the grasp during enough time to bring it to his mouth. Maintaining the glass was crucial as this sequence required the patient to keep attention highly focused on wrist extension. This was trained specifically with MI. This new coordination, called tenodesis, was learnt by combining both actual and mental practice. Data therefore suggest that MI practice contributed to successfully achieve the programming of new upper limb coordination. In this case, the new action has been built up without somesthetic feedback but probably with the help of visual feedback.

When the SCI is at the level of fifth cervical vertebra, the patient can no more extend his arm. Surgery may help to overcome this impairment with the solution of tendon transfer technique. As the biceps remains controlled voluntarily, the surgery consists in taking a part of the long biceps tendon to graft it onto the triceps tendon. Therefore, arm extension becomes possible again, although long and hard rehabilitation is required. To test the effectiveness of MI, Grangeon et al. (2010) implemented MI sessions into physical reeducation to a patient who underwent tendon transfer. Both sides were grafted (dominant arm first). The rehabilitation program was made of two distinct sessions, first the physical practice during a two-week-period, then the MI practice during the same duration. The reversed schedule was completed for the other arm. While the combination of MI and physical training significantly contributed to improve the kinematics of pointing a target place just in front of the patient, there was no order effect as performance of both sides did not differ one from another. MI was therefore believed to favor central networks reorganization after tendon transfer, thus making a part of a flexor muscle acting as an extensor. This result should be emphasized by analyzing the time course of cerebral activation changes along with the modification of muscle functions. Here again, MI might play a crucial role in the process of neuronal plasticity (Pascual-Leone et al., 1995). This could be favored and enhanced by determining the most efficient content for mental rehearsal. In a very-well documented review, Malouin and Richards (2010) recently proposed a set of rules for MI practice which should be followed by clinicians with the aim to elicit the most expected effect on motor rehabilitation. The authors advised that i) clinicians should be aware that it is imperative to evaluate MI ability before introducing mental practice, ii) patients who initially demonstrate difficulty in generating mental representation of movements eventually may improve their MI ability with repeated exposures, iii) the lack of somesthetic cues could perhaps be compensated for by additional visual information. With reference to this latter proposition, it should be mentioned that visual information could overcome the lack of information from the

body if it is correctly focused, i.e., on movement sequences that usually require sensory feedback from somesthetic information. In the conclusion section of their paper, Malouin and Richards (2010) underlined that researchers and clinicians should learn more about MI processes that will lead to define MI procedures and accurate clinical guidelines in greater details.

MI TREATMENT IN PATIENTS WITH PERIPHERAL INJURY (JOINT SPRAIN, MUSCLE TEAR, BONE FRACTURE)

Using MI with the aim to improve motor functions after peripheral injury is based on the same rationale as after central injury. There is however a strong difference between the two situations: The peripheral lesions are reversible and there is generally good hope of recovering motor function equivalent to that prior to trauma through surgery and rehabilitation, in all possible forms, including MI. However, using MI in rehabilitation programs after peripheral injury has not been often studied as for cerebral injury. Joint and muscle damages may also cause an immobilization of the patient. The longer it lasts, the more the rehabilitation may be extended and difficult. Again, mental rehearsal can contribute to recover simple movements (flexion, extension, internal or external rotation, abduction or adduction ...), but may also improve more complex motor programs. One of the first experiments addressing this issue is that from Newsom et al. (2003), although the experimental group included healthy participants. The study was conducted to examine the effects of MI on strength loss, after immobilization of the wrist. The experimental group was divided into two subgroups, the first performing mental practice during 2 weeks while the second completed a series of neutral activity which had nothing to do with mental activity and served as control group. Both groups showed force loss after immobilization by comparison to the maximal performance evaluated during the pre-test session. However, performance in the MI training group decreased to a lesser extent than in the control group. Results therefore revealed that MI may contribute to prevent strength loss that occurs during immobilization. Two other important studies may be briefly described to ascertain the role of MI in force gain. Firstly, Yue and Cole (1992) trained healthy participants with mental practice. Two other groups were studied, the control and physical training groups. The maximal isometric force of the abductor muscle of the fifth finger was measured before the participants mentally practiced the same movement. After a rehearsal period of 4-weeks (five sessions per week), the participants were re-tested. The isometric force significantly increased during the retention test by about 30% in the physical training group whereas the improvement was 22% in the imagery training group. As expected, the control group performance remained quite stable by improving 3.7% only. The authors concluded that strength increase could be achieved without repeated muscle activation. These force gains appeared to result from practice effects on central motor planning/programming. Almost the same result was obtained later by Ranganathan et al. (2004) although with additional useful results. The authors concluded that mental training enhanced the cortical output signal, thus driving muscles to higher activation level and increased strength. The important difference with Yue and Cole (1992) study was that a fourth group was trained on upper limb flexion with mental practice, i.e., biceps force. Training sessions lasted for 12 weeks (15 minutes per day, 5 days per

week). At the end of the training session, the group who trained the abductor of the fifth finger had increased its strength by about 35%. The elbow group increased the elbow flexion strength by 13.5% and the physical training group increased the finger abduction strength by 53% (the control group did not exhibit any change, as expected). Thus, force improved as a function of both muscle and training content. The rationale for explaining muscle strength improvement is the enhancement of cerebral motor command which was supposed to recruit more motoneurons to command muscle contraction and to better synchronize the motor units within the same muscle. This process must be qualified as a function of the cortical surface controlling each muscle. The five fingers are known to be represented by almost one third of the motor cortex while the cortical surface controlling the trunk and the lower limbs is about the same. Thus, improvement of muscle force by mental training is proportional to the cortical surface controlling this muscle. Keeping these results in mind, Guillot et al. (2009) tested the effect of MI training in patients with burnt hands. As well, Stenekes et al. (2009) investigated whether MI during the immobilization period after flexor tendon injury resulted in a faster recovery of central mechanisms of hand function. In parallel, Lebon et al. (2011) applied a MI program to participants after surgery of anterior cruciate ligament. Here, we have several experimental examples examining the variable 'type of muscle'.

First, the study by Guillot et al. (2009) aimed at investigating the effects of a 2-week MI training program combined with conventional rehabilitation on the recovery of motor functions in handed burn patients. Fourteen patients were randomly assigned to the imagery or the control group. Three complex manual motor sequences were tested at five intervals during a 5-week medical procedure. Range of motion during wrist extension, fingers dissociation and coordination were evaluated after the rehabilitation program. The results provided evidence that MI facilitated motor recovery, especially at the level of wrist movement. The study by Stenekes et al. (2009) brought more mitigated results. The patients were assigned to a mental training group based on kinesthetic MI of finger flexion movements during the post-operative dynamic splinting period. After the training period, the participants completed a reaction time test related to the preparation time of finger flexion, i.e., they should press a button as fast as possible following a visual stimulus. The other dependent variables were kinematics analysis of drawing, active total motion, and strength. After the immobilization period, the MI group reacted faster than the control group. However, no other significant influence of MI was evidenced on kinematics. As expected, MI positively influenced motor function, albeit with selective efficacy which should probably relate to the content of the mental program. As underlined by the authors, there are some factors that may have led to an underestimation of the MI effects, such as low patient compliance, suboptimal dosage of MI, no case control for injury severity, and small sample size. The MI content and the frequency of sessions training thus appear as being a major concern when testing MI efficacy and represent a key-problem that should be solved shortly. In any case, this is an important issue on which researchers should focus on in due course. The study by Lebon et al. (2011) aimed to assess the therapeutic effects of MI on the activation of lower limb muscles, as well as on the time course of functional recovery after surgery of the anterior cruciate ligament (ACL). Although the cortical surface controlling knee movement is reduced by comparison with upper-limb movement, this study exhibited positive outcomes from MI training. As a working hypothesis, we might assume that the issue related to the cortical surface controlling the muscle to be mentally trained could probably be overcome by the duration of training. Twelve patients with a torn ACL were randomly assigned to a MI or

control group and received usual sessions of physiotherapy. EMG activity of the quadriceps, anthropometrical data, and lower limb motor ability were measured throughout a 12-session-therapy. The data provided evidence that MI elicited greater muscle activation. The authors concluded that the muscle activation increase might originate from a redistribution of the central neuronal activity, as no anthropometric change was observed in lower limb muscles after MI practice. This study confirmed the effectiveness of integrating MI in a rehabilitation process by facilitating muscular properties recovery following motor impairment. MI may thus be considered a reliable adjunct therapy to help injured patients to recover motor functions after reconstructive surgery of ACL. The improvement remains nevertheless selectively focused on factors that are neurophysiological related. Lebon et al. (2011) did not evidence any change at the muscle level and the increase in muscle activation, with no change in muscle size, was attributed to central reorganization. In the same way, the results by Maddison et al. (2011) could be compared to those of Lebon et al. (2011). This imagery intervention improved knee laxity and healing-related neurobiological factors. No structural change was reported at the peripheral level (e.g., increase of muscle size or decrease in joint effusion), thus strengthening the hypothesis that MI influence is selectively confined to central nervous system.

Training schedules integrating MI are believed to keep the motor programs into memory, due to brain plasticity (Pascual-Leone et al., 1995). This contribution should be emphasized in rehabilitation periods during which actual practice is not yet possible, due to immobilization or pain. More precisely, the synchronization of motor units is thought as being the main process improved by mental training, thus preserving, at least partially, muscle strength. Motor units synchronization itself is probably dependent upon central reorganization of cerebral networks controlling movements which are mentally rehearsed. Therefore, if actual practice is needed to build up and store motor programs into long term memory, MI may also play a crucial role in these operations. Due to its intrinsic properties, MI should also have the potential to be beneficial for the elderly. This is a specific population which may be particularly affected by the maintenance of motor function with advancing age. As a consequence of ageing, muscle size decreases as along with range of motion, and such processes may be compounded by the reduction of general motor activity. Postural functions and locomotion may be significantly impaired, thus increasing the risk of falling. Hamel and Lajoie (2005) reported a beneficial effect of MI practice with decrease in both attentional demands and postural oscillations on the antero-posterior axis. The use of a tailored mental practice should therefore enable regular maintenance and preservation of the mobility in the elderly, especially as MI quality evolves with age. Interestingly, Malouin et al. (2010) showed that MI alterations are associated with an age-related decline in visuo-spatial and kinesthetic working memory. MI is thus a good way in maintaining motor potential but also one's own ability to form mental images.

GENERAL CONCLUSION

To conclude, we must first reiterate that MI is not a substitute of the classical course of physical therapy during the rehabilitation process, but rather an adjunction which may bring additional benefits, e.g., among others, saving time and/or improving motor function. This is

an attractive and cost-effective task requiring the patient to engage himself voluntarily and actively in this process. It is actually important to involve patients in the rehabilitation process by making them active. MI is a rather good candidate for that. As a specific cognitive operation, MI remains independent upon residual motor function after central or peripheral injury. Most patients with stroke and patients with peripheral injury should thus be able to use MI of movement that can no longer be physically executed (nevertheless, depending upon their own abilities to form and manipulate mental images). Some periods could be favorable for starting short sessions of MI rehabilitation, e.g., just after the acute phase of stroke or after joint surgery. As previously suggested, MI could be here designed to maintain motivation and manage stress, using positive mental images. Nothing is usually done for rehabilitation within this period, and MI should enable early therapeutic interventions when other rehabilitative treatments remain impossible. In parallel, we also should solve some remaining issues related to MI practice, e.g., the onset of neuropathic pain in a substantial proportion of people with SCI when performing MI (Gustin et al., 2008). We must also improve methods and tools enabling the clinicians to follow-up and evaluate the quality and efficacy of the MI work. We recently proposed an index of MI quality integrating different sub-indices (Collet et al., 2011), including questionnaires and interviews, mental chronometry and several physiological parameters measuring i) the functional state of the patient (physiological activation) and its compliance with mental practice requirements, and ii) the ability to maintain attention on motor rehearsal across time, with the aim to individually determine the exact amount of MI trials that should be programmed with the highest efficiency (electrodermal and heart rate responses being recorded during mental work).

Defining more extensively the general conditions and the rules of MI practice to ensure the effectiveness of MI is one of the most important issues. First, we should define criteria to select the patients who would be most likely to benefit from mental training. Examining the best way to use MI and adjusting its content to the patients' features will certainly be a main focus of research in coming years. Schuster et al. (2011) reviewed the main MI contents that were applied to patients with motor impairment. While the goal of each experimental design was very similar, i.e., selecting the conditions that are likely to provide the best motor improvement, we note the high diversity among programs, both in their content and their frequency of use. Malouin and Richards (2010) recently addressed a series of issues related to how MI should be scheduled. Should patients be lying down and relaxed while listening to the instructions, or, in contrast, should they be more actively engaged in their training? Should they learn to self-monitor their training or being kept under clinical supervision? Despite these outstanding issues, the experimental data described in this review support the hypothesis that MI is a valuable and promising cost-effective technique to enhance recovery after motor impairment.

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Chapter 45

NEUROPSYCHOLOGICAL ISSUES IN STROKE REHABILITATION

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INTRODUCTION

Neuropsychologists perform an important function in the evaluation and treatment of patients who have experienced strokes. The role the neuropsychologist plays with patients and their families varies according to the type and severity of stroke and the patient's current position in the continuum of health-care (e.g., from the acute-care hospital to the inpatient rehabilitation unit/center to the outpatient clinic). Neuropsychologists make contributions to the rehabilitation of stroke patients through activities such as neuropsychological evaluations (ranging from brief bedside assessments to comprehensive multi-hour evaluations), interventions to address the cognitive and emotional impact of stroke, and consultation with the patient, patient's family, and treatment team in defining, accepting, and working toward realistic goals for the rehabilitation process.

Assessment of neurocognitive functioning in stroke patients is crucial as the incidence of cognitive dysfunction can be as high as 70% in patients with recent strokes (Ruchinskas, Singer, and Repetz, 2000; Mast, MacNeill, and Lichtenberg, 1999; Tatemichi, et al., 1994). Even "pure motor" strokes, or those with little evident cognitive impact, will show deficits when properly assessed with a neuropsychological battery (Hinkle, 2002). In addition, the neuropsychological understanding of brain-behavior relationships can assist the patient and treatment team in understanding and conceptualizing changes in cognitive, emotional, and/or behavioral functioning following stroke. This contribution is not dependent on giving a particular test or obtaining a particular evaluation result but instead comes from the neuropsychologist's educational background and ability to assist with comprehensive case conceptualization. Finally, although not a focus of this chapter, the neuropsychologist

contributes much to the overall success of the rehabilitation program by aiding the patient, patient's family, and the treatment team in dealing with the emotional aspects of stroke. This contribution, which the neuropsychologist is uniquely suited to make, is as important as the assessment and intervention process for the cognitive difficulties we discuss below. The neuropsychologist's knowledge base concerning the mechanisms, sequelae, pathology, and outcome of strokes greatly aids in the assessment and treatment of stroke patients in rehabilitation setting and leads to improved patient care.

Although this review covers many of the disorders that can be associated with strokes, it is rare that these conditions are found in isolation. Part of this fact is because strokes are not constrained to any one particular vascular territory or brain region. Each stroke is unique. Another reason for the decreased chance of encountering pure agnosia, apraxia, and visual disorders is due to the nature of rehabilitation admissions and reimbursement. Since walking is often the "gold standard" in terms of deficits that can lead to a rehabilitation admission, disorders that involve little motor weakness or solely upper extremity deficits will have a lesser chance of admission (and subsequent evaluation by the clinician). Hence, while we focus this review on discrete conditions commonly associated with strokes, the neuropsychologist will typically encounter a single patient with aspects of two or more of the syndromes discussed below, as the more neurologically involved patients are more likely to gain admission.

DEFINITIONS AND EPIDEMIOLOGY

Stroke can be defined as the "sudden occurrence of a nonconvulsive, focal neurologic deficit" (Victor and Ropper, 2001, p. 823). The World Health Organization defines stroke as an "acute neurological dysfunction of vascular origin with sudden (within seconds) or at least rapid (within hours) occurrence of symptoms and signs corresponding to the involvement of focal areas of the brain" (World Health Organization, 1989, p. 1412). Although other terms are used to describe this event at times (apoplexy, cerebrovascular accidents [CVA], or shock) stroke is the preferred term (Victor and Ropper, 2001).

Strokes are the most frequent type of cerebrovascular disease. Between 500,000–700,000 persons in the US have a stroke each year (Weinstein and Swenson, 1998). Over three million people in the US experience disability as the result of stroke. Stroke is the third most common cause of death in the US. Although strokes typically occur in those sixty-five years of age and older, twenty-five percent of strokes affect people aged forty-five to sixty-five. During a stroke, reduced blood flow limits the supply of nutrients provided to brain cells, primarily oxygen and glucose. This reduction in nutrient supply leads to cerebral ischemia when blood flow is not sufficient to maintain the cell and to cerebral infarction when the reduced blood flow leads to cell death (Victor and Ropper, 2001).

TYPES OF STROKE

There are two broad categories of stroke: obstructive and hemorrhagic. Obstructive strokes are due to blockages in the cerebral arteries and are of two primary types: thrombotic

and embolic. In thrombotic strokes, atherosclerotic plaques or fat deposits gradually develop within the arteries over time and this leads to narrowing of the vessel, which in turn reduces blood flow. In this type of stroke, symptoms typically develop over several hours to a few days at times. This type of stroke usually affects the middle cerebral arteries and vertebrobasilar arteries (Weinstein and Swenson, 1998). Cerebral atherosclerosis, or narrowing of the blood vessel, is the most common cause of ischemia and infarction. Thrombotic strokes account for approximately 60 percent of all strokes (Victor and Ropper, 2001).

During embolic strokes, material breaks away from an artery wall or foreign matter finds its way into the cerebral arteries and then becomes lodged in the vessel. Approximately twenty percent of all strokes are embolic, with roughly half of them originating from cardiac sources. Taken together, thrombotic and embolic strokes account for over 80% of first strokes (Terent, 1993). In contrast, in hemorrhagic strokes, the blood vessel bleeds onto the brain tissue. Typically, with hemorrhagic strokes, the vessel ruptures and there is the sudden onset of symptoms. Primary intracerebral hemorrhage is the third most common cause of stroke (after thrombotic and embolic strokes) and is most often due to hypertension. Intracerebral hemorrhages account for approximately 11% of first strokes (Caplan and Moelter, 2000).

Subarachnoid hemorrhage is the fourth most common type of stroke. Subarachnoid hemorrhages are typically caused by a ruptured aneurysm. However, other sources, such as arteriovenous malformations, which are congenitally abnormal blood vessels with abnormal blood flow that are susceptible to leakage, are less commonly the cause of hemorrhagic stroke. Subarachnoid hemorrhages account for approximately 6% of hemorrhagic strokes (Victor and Ropper, 2001).

Compared to obstructive strokes, hemorrhagic strokes are much more fatal in the short term. During the first month following stroke, 52% of persons with intracerebral hemorrhage will die, 45% of persons with subarachnoid hemorrhages will die, and 10% of persons with thrombotic-embolic strokes will die. The long-term outcome of obstructive and hemorrhagic strokes is similar, despite the differences in early mortality and incidence of these two types of strokes (Bamford, Dennis, Sandercock, Burn, and Warlow, 1990).

Transient ischemic attacks (TIAs) differ from strokes in that the symptoms produced by TIAs do not persist and are typically reversed in minutes to hours. In TIAs, the episodes of impairment typically last up to 15 minutes and fully resolve within one day. Approximately one-third of persons who experience a TIA will ultimately experience a stroke (Lezak, Howieson, Loring, Hannay, and Fisher, 2004). Reversible ischemic neurologic deficits (RINDs) are similar to TIAs in that the symptoms are transient and fully resolve. However, the symptoms in RINDs last from one day up to 3 weeks, versus the one-day resolution of symptoms in the TIAs.

MAJOR ARTERIES/BLOOD SUPPLY TO THE BRAIN

Two carotid arteries and two vertebral arteries irrigate the brain. The carotid system provides blood for the frontal and parietal lobes, two-thirds of the temporal lobes, hypothalamus, basal ganglia, and eyes. The internal carotid arteries' major branches are the anterior cerebral artery and middle cerebral artery. These arteries provide blood to anterior

and middle portions of brain. The middle cerebral artery is largest supplier of blood to the cerebrum. The minor branches of the internal carotid arteries include the ophthalmic artery, anterior choroidal artery, posterior communicating artery, and anterior communicating artery. These minor branches of the internal carotid artery provide blood to various subcortical regions.

The vertebral system supplies the posterior temporal lobe, occipital lobe, and upper part of spinal cord, brainstem, midbrain, thalamus, cerebellum, and inner ear (Joseph, 1990). The vertebral arteries join to form the basilar artery. The major branches of the vertebral arteries are the basilar artery and posterior cerebral artery and they supply parts of the temporal and occipital lobes. The minor branches of the vertebral arteries (the anterior and posterior inferior cerebellar arteries) interconnect to form the circle of Willis. This formation helps the brain compensate if blockage of vertebral or carotid artery occurs on one side of the brain.

REGIONAL SYNDROMES FOLLOWING STROKE

A stroke limited to the anterior cerebral artery is rare. Symptoms seen with this type stroke are usually part of the symptomatology of middle cerebral artery strokes. Anterior cerebral artery stroke symptoms include contralateral lower extremity motor impairment with relative sparing of upper extremity functioning. Other symptoms may include incontinence, motor aphasia, and behavioral/affective changes.

The middle cerebral artery has the largest distribution area of any cerebral artery. Symptoms following middle cerebral artery strokes typically include contralateral hemiplegia of face, arms, and legs; hemisensory deficits; and loss of vision in the visual field contralateral to the lesion. Various forms of aphasia may occur following a left hemisphere middle cerebral artery stroke. Typical symptoms following a right hemisphere middle cerebral artery stroke include visual-spatial and constructional deficits, impaired attentional processes, unawareness of deficits, and impaired understanding of emotional components of language. Middle cerebral artery strokes usually are more rapid in onset and result in greater disability than other types of strokes. Visual field cuts following middle cerebral artery strokes are common.

Internal carotid artery strokes pose the greatest risk to watershed regions (border-zone) of brain irrigation. Deficits depend of the part of the artery most affected although one usually sees effects similar to middle cerebral artery strokes. Posterior cerebral artery strokes typically result in sensory loss, oculomotor palsy, and cerebellar ataxia. Cortical aspects of posterior cerebral artery strokes may include loss of color vision, tunnel vision, alexia without agraphia, and memory impairment.

APHASIA

Aphasia is an acquired impairment of language (speech, writing, and/or reading) due to an injury to the brain (for reviews, see Beeson and Rapcsak, 1998; Kolb and Whishaw, 2003; Benson, 1993). Aphasia (sometimes referred to as dysphasia) is typically produced by left hemisphere damage. Aphasia is a central language disorder and affects not only spoken

language but also, for the majority of cases, writing and reading. Paraphasic errors, which are errors leading to sound substitutions and incorrect word choices, are common features of aphasia. Within the area of language impairment, agraphia refers to a writing impairment and alexia to a reading impairment.

Because approximately 95% of right-handed and 70% of left-handed people are left hemisphere dominant for language, aphasia typically results from a lesion to the left hemisphere. Aphasia may result from right hemisphere damage for left-handed persons. Crossed aphasia is a rare condition in which right-handed people develop aphasia following right hemisphere damage.

In categorizing aphasias, a useful framework is to divide aphasias into fluent aphasias versus nonfluent aphasias. Within each category there are several types. In the aphasias discussed below, the lesion location thought to be important in the production of aphasia syndromes will be highlighted. For a recent review of the anatomy of aphasia, please see Kreisler et al. (2000).

Fluent Aphasia

The fluent aphasias are characterized by fluent speech production in conversational speech, although there can be significant paraphasias. One type of fluent aphasia is Wernicke's aphasia; also known as "sensory aphasia". In this type of aphasia, neologisms and paraphasias characterize the fluent speech output that is produced. Impairments are demonstrated in written and auditory comprehension as well as repetition. Impairment is also usually present in naming, and cueing for naming is rarely of benefit. In this type of aphasia, speech output may be meaningless and the term "jargon aphasia" may be used to describe it. Typically large posterior superior temporal gyrus lesions produce this type aphasia (Benson, 1993).

Another type of fluent aphasia is transcortical sensory aphasia. In this type of aphasia, verbal output is fluent but paraphasias are frequent. Language comprehension is impaired but repetition is intact. Echolalia is a common feature of this type aphasia. Naming, writing, and reading are also impaired. Lesions in the parietal and temporal areas that are posterior to the perisylvian region typically produce this type aphasia.

A third type of fluent aphasia is conduction aphasia. In this type of aphasia, speech output is fluent but is characterized by paraphasias. Comprehension is relatively intact, although there is a significant impairment in repetition and object naming. Lesions producing this type aphasia are often below the supramarginal gyrus in the white matter (arcuate fasciculus), although there may be lesions outside this area. The lesions disconnect intact motor speech areas from intact comprehension areas.

A fourth type of fluent aphasia is anomic aphasia. The distinguishing feature of this type aphasia is severely impaired naming. Speech output is fluent and little or no paraphasias are present. Both repetition and comprehension remain intact. Empty but lengthy verbal output is noted. The lesion is often in the angular gyrus or inferior temporal regions but the lesion may also be found anywhere in language area and even in right hemisphere at times. In this type of aphasia, noun-finding deficits typically originate from damage in the temporal cortex while verb-finding deficits typically are caused by left frontal injuries.

Nonfluent Aphasia

Nonfluent aphasia is characterized by difficulty with the ease/fluency of speech output. As with fluent aphasia, there are several types of nonfluent aphasia. Broca's aphasia, also known as motor or expressive aphasia is characterized by nonfluent speech output with relatively intact comprehension. Repetition skills are impaired but not as impaired as conversational expression. In this condition, although object naming is poor, cueing provides a significant benefit. This was the first recognized aphasia syndrome. Lesions producing this type aphasia are typically in the superior division of the middle cerebral artery in the dominant frontal region with at times lesion extension into the basal ganglia.

A second type of nonfluent aphasia is transcortical motor aphasia. In this type aphasia, verbal output is nonfluent but echolalia can occur. Both comprehension and repetition are generally adequate. Persons with this type aphasia typically do not attempt to spontaneously produce speech. Naming is relatively intact. Damage in the frontal/prefrontal region of the dominant cerebral hemisphere is typically involved, either superior or anterior to Broca's area and often in the watershed zone between the anterior and middle cerebral arteries. In supplementary motor area aphasia, the language deficits produced are identical to those produced in transcortical motor aphasia. However, the lesion that produces this type aphasia is in the dominant medial frontal area.

Another variant of nonfluent aphasia is mixed transcortical aphasia, also known as "isolation syndrome". In this type of aphasia, speech production is nonfluent and conversational speech is similar to Broca's aphasia. Comprehension is also impaired but repetition is relatively intact. Naming is also impaired. The lesion that produces this type aphasia is usually diffuse or multifocal. This results in the isolation of the perisylvian language zone from the surrounding cortical areas.

A final type of nonfluent aphasia is global aphasia. In this type aphasia, all language components are impaired. This includes speech production/output, comprehension, repetition, reading, writing, and naming. Lesions producing this type aphasia are extensive and typically there is involvement of the entire perisylvian language zone. In global aphasia, even if the patient receives long-term speech therapy, the person is rarely able to recover useable language functioning (Strub and Black, 1988).

Pure Aphasia

In addition to the fluent and nonfluent aphasia discussed above, there are also some conditions described as "pure" aphasia. In these conditions, only a specific language function is impaired. One type of pure aphasia is alexia without agraphia. This represents an impairment in reading in the absence of other symptoms of aphasia. This condition is typically produced by left occipital infarctions in the territory of the posterior cerebral artery.

A second type of pure aphasia is pure word deafness. In this condition, although reading, spontaneous speech, and naming are intact, there is impaired comprehension for spoken language and impaired repetition. This is a rare syndrome that typically results from bilateral temporal lobe lesions or a unilateral deep left temporal lobe lesion.

In addition to the cortical aphasia described above, aphasia can also result from subcortical damage. In subcortical aphasia, the onset of symptoms is acute and the patient is

typically mute. In this condition, the patient usually recovers to the point of slow speech output that is poorly articulated and paraphasic with repetition intact. Subcortical aphasia often resembles the transcortical aphasia because of the preserved repetition. Other language features vary widely and there is a tendency for this type of aphasia to fully resolve. This condition includes thalamic aphasia and syndromes associated with damage to basal ganglia and white matter pathways (Crosson, 1992).

Although the above discussion of aphasia focuses on left hemisphere lesions producing aphasia syndromes, the right hemisphere (nondominant hemisphere) also contributes to language function. The right hemisphere appears to have reasonably good auditory comprehension of language as well as some involvement in the spatial components of reading and writing. However, the right hemisphere typically involves little or no speech output. Although the right hemisphere is able to recognize words, it is not able to understand grammar rules and structure or the so-called “pragmatic” aspects of speech (Kolb and Whishaw, 2003). In addition, the right hemisphere appears to play a part in “how” speech is expressed that contrasts the left hemisphere’s focus on “what” is spoken. In fact, Mesulam (2000) argues for a right hemisphere system that parallels the aphasic disorders discussed above, with the expression and reception of prosody, emotional valence, and humor as the basis for the disturbance. Because of a reduction in prosody, individuals with right hemisphere deficits often have monotone verbal production that may be inaccurately interpreted by staff members and/or family members as reflective of depressed mood.

Assessment of Aphasia and Recovery

During the acute phase of stroke treatment, assessment is typically limited to bedside evaluations. These evaluations should focus on conversational speech production, comprehension for auditory material, repetition, and naming as well as a brief assessment of reading and writing. Often, this type of evaluation must be completed at the bedside through brief screening tests due to the impairments in comprehension or expression that accompany acute strokes.

There are several standardized tests for aphasia but they are not designed for bedside use. These more comprehensive assessment batteries are best utilized once the patient is no longer in the acute stage of injury. Included in the list of more comprehensive aphasia test batteries are the Boston Diagnostic Aphasia Examination (Goodglass and Kaplan, 2001), Western Aphasia Battery (Kertesz, 1982), Multilingual Aphasia Examination (Benton, deHamsher, and Siven, 1994), and Aphasia Diagnostic Profiles (Helm-Estabrooks, 1992). There are some single domain tests that are also quite useful in aphasia assessment. These include the Boston Naming Test (Kaplan, Goodglass, and Weintraub, 2001) for naming, the Token Test from the Multilingual Aphasia Examination for comprehension, and the Psycholinguistic Assessment of Language Processing in Aphasia (Kay, Lesser, and Coulthart, 1992) for reading and writing. For a thorough discussion of the variety of measures that can be used both during the acute and post-acute phase in aphasia assessment, please see Lezak et al. (2004) as well as other pertinent chapters in this volume.

During the first month after stroke, significant spontaneous recovery typically occurs with ongoing but less dramatic recovery occurring for several months to approximately a year. Spontaneous recovery of comprehension is typically more complete than spontaneous

recovery of speech output (Benson, 1993). It has typically been thought that most recovery from aphasia occurred by one year after stroke. However, recent research indicates that new treatments that involve motor activation theories (similar to constraint induced movement therapy) can provide significant benefit in aphasia recovery even for patients who are several years post-stroke (Pulvermuller et al., 2001).

In the inpatient rehab setting, interventions to address aphasia are primarily conducted by the speech-language pathologist. The speech therapist may use a variety of remediation and compensation strategies to address targeted aphasia deficits. Aphasia treatment beginning in the acute phase of recovery results in nearly twice the beneficial effect seen solely from spontaneous recovery. Treatment that begins after the acute phase has passed achieves a smaller, but notable effect. Therapeutic interventions designed to improve speech output are typically more successful than those designed to improve comprehension (Albert, 1998). Various strategies are used in aphasia treatment. These include output-focused therapy, melodic intonation therapy, psycholinguistic approaches, cognitive neurorehabilitation, and computer aided therapy. Many aphasias tend to ultimately evolve into some degree of an anomic aphasia, which is not surprising given the large perisylvian areas that have been implicated in the process of confrontational naming.

The less severe forms of aphasia discussed above may not be seen in the inpatient rehabilitation setting because the physical deficits produced from such lesions may not be of the magnitude to produce the physical impairments necessary to qualify for inpatient rehabilitation treatment. In working with aphasic patients in the inpatient rehabilitation setting it is important to work in coordination with the therapists, especially the speech therapists, to insure consistency of approach in allowing the patient to communicate by the most effective means possible. Even when the patient has significant aphasia, it is often possible to develop communication strategies with the patient that will allow for significant information exchange to occur. This not only helps the examiner determine more broadly the cognitive and emotional impact of stroke on the patient, it also helps the patient increase his/her sense of comfort and control knowing that his/her needs and desires can be communicated and understood. Patients can be severely aphasic but appear quite functional in terms of their rehabilitation therapies. Close observation and assessment usually reveals that such individuals are quite adept at picking up on visual and prosodic cues ("I know that they are commanding me to do something over there"). Both the exact level of comprehension and the compensation techniques utilized are essential to communicate to staff and family to help them understand exactly what can and cannot be communicated to the aphasic patient.

HEMIPARESIS AND OTHER MOTORIC DISORDERS

Muscle weakness, discoordination, and/or some degree of paresis are often seen in stroke patients. As the criteria for admission to inpatient rehabilitation often requires motor system dysfunction, the practicing neuropsychologist should be familiar with the causes and effects of hemiparesis and other motoric disorders. Some degree of hemiparesis can accompany lesions that occur anywhere along the corticospinal and corticobulbar tracts in the brain. Thus, damage to the brainstem, pons, midbrain area, white matter tracts of the subcortex, thalamus, basal ganglia, cerebellum, and motor and parietal cortices can all cause paresis

(Victor and Ropper, 2001). In addition, damage to supplementary motor cortices, while not causing paralysis, *per se*, can greatly interfere with the initiation and planning of motor movements. Luria (1966) described a wide variety of initiation and imitation tasks to assess motor planning skills, praxis, and the ability of other behavioral systems (vision, speech) to aid or hinder the accurate performance of motor movements. Luria suggested that damage to the premotor regions would cause both motor incoordination and “efferent motor” perseveration, secondary to disinhibition of primitive automatic movements. The degree of disturbance in a patient’s ability to switch smoothly from one motor movement to another was taken as a sign of the extent of cortical damage. Malloy, Webster, and Russell (1985) found that perseveration on Lurian motor tasks, while occurring more frequently in patients with frontal lesions, also was quite common in individuals with posterior lesions. Ruchinskas and Giuliano (2003) also found that the presence of motor incoordination and perseveration after stroke were quite common and more indicative of global brain integrity than of damage to any particular cortical area, *per se*. At the other end of the spectrum, damage to some areas, particularly the frontal cortex and the caudate region in the subcortex, can cause a lack of spontaneous motor movements (akinesia) in the presence of relatively preserved motor strength (Kumral, Evyapan, and Balkir, 1999). We have observed individuals who will not actively move a limb on command, but will show spontaneous (and purposeful) movement in more unplanned or reflexive actions (e.g., catching a rubber ball being thrown to them). Akinesia also can be present in neglect syndromes and must also be distinguished from the apraxic syndromes listed below.

Perhaps the most effective treatment of any disorders that are discussed in this chapter falls within the arena of the treatment of hemiparesis. Constraint-induced movement therapy, a treatment where the unaffected limb is restrained and a series of shaping paradigms are used to reduce the learned nonuse of the affected (usually upper) extremity, has shown tremendous promise in the rehabilitation literature and in practice (Taub and Uswatte, 2003). While the original design was for long periods of restraint (i.e., 6 hours or more per day), some research has suggested benefit from shorter duration (3 hours per day) of this treatment (Sterr et al., 2002). Taub (2004) reports extension of this treatment to other chronic disabling conditions such as weakness after traumatic brain injury, cerebral palsy, and spinal cord injury. While originally designed for the treatment of nonuse of an extremity after one year, this technique is increasingly being used in acute stroke settings and even as part of home therapy programs, although the efficacy research on which to base these practices is small (Pierce et al., 2003; Dromerick, Edwards, and Hahn, 2000).

APRAXIA

Apraxia is an acquired disorder that negatively impacts the ability to complete learned movements and motor sequences. The deficits in apraxia are not due to impairments in sensation, coordination, strength, or lack of comprehension. Although the types of tasks used to assess the various forms of apraxia will be discussed below, it is important that the patient receives a thorough examination to insure other motor, sensory, language, or cognitive problems are not causing the observed dysfunction. Deficits seen in apraxia are considered high-level motor disturbances that are due to problems with motor planning and in completing the integrative steps that are necessary to complete skilled movements. Apraxia

negatively affects the ability to execute and select changes in body position. In order to complete skilled movements correctly, both conceptual knowledge and production knowledge are required (Heilman, Watson, and Gonzalez-Rothi, 1998).

Control of praxic movements in right-handed people is in the left (language dominant) hemisphere. In left-handed people, the right hemisphere usually controls praxic movements, even when language dominance is on the left. Handedness is more closely related to skilled movement than is language dominance (Strub and Black, 1988). In right-handed people, apraxia is almost never produced by right hemisphere lesions (Heilman and Gonzalez-Rothi, 1993). Damage to subcortical structures may also be necessary for the expression of marked apraxia. For example, a recent study suggests that cortical and subcortical structures provide differential contribution to praxis and the deficits seen in apraxia (Hanna-Pladdy, Heilman, and Foundas, 2001).

Praxis is conceptualized along a continuum of difficulty in performing skilled motor tasks. Completing the tasks to only verbal command is the most difficult. This is done without giving nonverbal cues and without using the object itself. If the patient cannot complete the action following verbal command, then the examiner can attempt to have the patient complete the action by using imitation. Using the real object itself provides the patient with the least difficult scenario.

In theory, performing the action to command only involves the dominant hemisphere's language function. This information then travels to the supramarginal gyrus. Words are subsequently associated with their kinesthetic memories in the parietal cortex. Then these sensory memories are transferred to the premotor area, which ultimately directs the information to the motor strip, and the action is completed. Ideomotor apraxia can be caused by a lesion anywhere along this circuit. This neural circuit is for apraxia in the right hand. Apraxia in the left hand is regulated by information being transferred from the premotor area on the left to the right premotor area via the corpus callosum. A lesion in this circuit will produce left-hand apraxia, often called sympathetic dyspraxia (Strub and Black, 1993).

Another form of apraxia is buccofacial or oral apraxia. In this type of apraxia, the patient demonstrates an inability to complete skilled movements of the speech apparatus and face. One way to assess for this type of apraxia is to ask the patient to blow out a match or drink from a straw. Verbal or oral-motor apraxia occurs when oral apraxia negatively impacts the ability to produce speech sounds.

There are several types of limb apraxia, which is the inability to perform learned movements with the upper extremities. In limb kinetic apraxia, the patient is unable to make precise individual finger movements. The impairment is typically seen in the hand contralateral to the brain lesion. This may be assessed by asking the patient to pick up a dime from a flat surface. In addition, the Grooved Pegboard Test can be used to help assess this skill (Heilman et al., 1998).

Ideomotor apraxia is the most common type of apraxia. Ideomotor apraxia is caused by a disruption in the praxis production system. In this type of apraxia, errors are most frequently seen when the patient attempts to pantomime transitive (using a tool or instrument) acts to command. In patients with this type apraxia, performance usually improves when patients are allowed to imitate the action. The errors here are primarily spatial and temporal errors. Both perseverative errors and sequencing errors are common. Lesions producing this type of apraxia in right-handed people are usually in the left hemisphere and vice versa and may be in

multiple areas including the corpus callosum, basal ganglia, supplemental motor area, and inferior parietal lobe (Strub and Black, 1993).

In conduction apraxia the patient demonstrates more impairment when trying to imitate action than when asked to pantomime the action based on verbal command. This is caused by a disconnection between the input and output praxicons. The exact location of the lesion that produces this type of apraxia is unknown (Heilman et al., 1998).

In dissociation apraxia, the use of objects themselves and imitation of actions is intact, but the patient is impaired when asked to pantomime an action. Disruption of information passing through the corpus callosum is likely responsible for the production of this type of apraxia. Right-handed patients may demonstrate a combination of dissociation apraxia and ideomotor apraxia following callosal lesions. Left-handed patients may demonstrate ideomotor apraxia without aphasia following right hemisphere lesions (Heilman et al., 1998).

Ideational apraxia results in the patient being unable to formulate an ideational plan for carrying out a series of steps involved in an action. Impairments are noted in the ability to sequence the actions in the proper order. This type of apraxia is caused by difficulty in complex motor planning that is of a higher-order nature than that involved with ideomotor apraxia. Each step of the sequence can be performed in isolation but the person cannot grasp the overall concept and is unable to integrate the parts into complete sequences. The examiner can assess this type apraxia by asking patient to complete a series of actions such as opening a tube of toothpaste, taking a toothbrush from the holder, and placing toothpaste on the toothbrush.

Constructional apraxia occurs when the patient has impairment in the conceptual knowledge needed to select and use objects and tools. The patient demonstrates an inability to recall the types of actions for specific objects and tools. The person with constructional apraxia may have impaired mechanical knowledge, may be unable to describe the function of tools, and/or may be unable to recall which tool goes with which object. These type representations are usually lateralized contralateral to the preferred hand in the temporoparietal junction.

As indicated above, there are several specific tasks that can be used to assess apraxia. One type of assessment task is asking the patient to gesture to command. It is important to include both transitive (using a tool and instrument) and intransitive (communicating gestures) conditions when requesting the patient complete the gesture to command. Another type is gesture imitation. In gesture imitation tasks, the patient should also be asked to gesture in response to seeing and holding tools and objects. Pantomime recognition and discrimination is another type of assessment task. Also, asking the patient to complete sequential acts, such as making a sandwich. In addition, the patient can be asked to demonstrate the association tool-object knowledge and mechanical knowledge.

Stroke patients often are not aware of their apraxic deficits and attribute these problems to inexperience using their left arm or right hemiparesis. Activities of daily living are often impaired in the presence of ideational, ideomotor, and conceptual apraxia. Spontaneous recovery from apraxia for right-handed patients may be due to the right hemisphere ability to develop skilled motor movement support. Surprisingly, despite the prevalence of apraxia in rehabilitation settings, little has been written about systematic efforts for the treatment of limb apraxias. For the most part, reports have consisted of case-series designs (e.g., Smania, Girardi, Domenicali, Loar, and Aglioti, 2000) as opposed to evaluation of actual apraxia treatment strategies (e.g., van Heugten et al., 1998). The results, as a whole, do suggest that

apraxia treatment in stroke patients, in the form of deficit remediation or compensation, can be beneficial in increasing the patient's functional independence (van Heugten et al., 1998). In general, therapeutic efforts target tasks that resemble as closely as possible tasks the patient will face in her/her normal daily routine (Goldenberg, Daumuller, and Hagmann, 2001).

Part of the difficulty in evaluating and treating apraxia, as with many of the disorders listed in this chapter, is that these symptoms often co-exist with other cognitive disorders. For example, a patient recently presented with a left hemisphere stroke that resulted in right hemiparesis and moderate deficits in naming, comprehension, fluency, and repetition (and other cognitive difficulties). She had obvious difficulties in planning motor activities, but the treatment team was not fully able to ascribe the extent of these difficulties to praxis versus language deficits. The patient returned to the rehabilitation unit three months later for deconditioning after pneumonia. At that point she had minor difficulties in naming as the only visible cognitive residual of the stroke, yet it was much easier to see the extent of her apraxia: when she attempted to brush her teeth, after looking confused for several seconds, she raised the toothpaste tube to her lips as if it was a toothbrush.

NEGLECT

Unilateral neglect is often conceptualized as a failure to report, attend to, orient toward, or respond to stimuli presented in a contralateral side of space that cannot be explained by sensory or motor dysfunction (Heilman and Valenstein, 2003). Neglect is probably one of the more frequently encountered conditions listed in this chapter, but one of the least understood. Often missed, particularly in its subtlest forms, or confused with other sensory deficits (e.g., a visual field cut), neglect can be a source of tremendous disability in daily life and a significant challenge to the rehabilitation team. While often simplified to merely a "visual" or "attentional" phenomenon, neglect is actually a multi-faceted disturbance of attention, perception, and/or action (Buxbaum et al., 2004).

Neglect is typically thought of as a non-dominant hemisphere phenomena, meaning that in the majority of individuals this syndrome involves the lack of attention to the left side of hemispace. Right-sided neglect also occurs, although traditional wisdom suggests decreased severity and faster recovery when compared to left-sided neglect. Still, some studies have posited that right-sided neglect occurs, in one form or another, in up to 40% of individuals who have suffered a stroke in their left hemisphere (Beis et al., 2004), indicating that evaluation of these functions regardless of stroke location should take place. Similar to aphasia, various brain structures and regions can be affected and subsequently produce neglect. While the region most often associated with neglect is the right parietal lobe, damage to the thalamus, basal ganglia, and frontal lobe can also produce neglect (Pierce and Buxbaum, 2002).

Neglect, like aphasia, is not a unitary syndrome. Variations can be seen in the severity and type of neglect. In its mildest form, neglect can be seen in extinction of sensation on the affected side when undergoing bilateral sensory stimulation (e.g., the patient will only report feeling right sided stimulation when both hands were touched). As such, the patient may not appear to the treatment team to have any deficits until they are challenged, such as placed in a busy atmosphere with multiple competing stimuli, whereby the manifestations of neglect may

be seen. In more moderate cases of neglect, the inattention to contralateral space becomes quite obvious to observers. It has been well established that the presence of neglect corresponds to poorer rehabilitation outcome than if neglect was not present (Buxbaum et al., 2004). Potential progress in rehabilitation can thus be gauged early on in the inpatient stay by engaging the patient in tasks of orientation to the ignored hemispace and judging how much external cuing it takes to draw the patient's attention to the target. Those who need minimal cuing obviously have a better prognosis for a shorter stay and more functional recovery than those who will never orient far enough to perceive the target stimuli. Perhaps the most severe cases of neglect are in individuals who not only have a severe neglect but also have frank anosognosia regarding their deficit (or, in some cases fail to recognize their contralesional extremities as their own). While some degree of unawareness of deficit occurs in up to 50% of patients with neglect (Gianella and Mattioli, 1992), such gross disregard (often accompanied by confabulation) of deficits is usually associated with large lesions and significant hemiparesis (Leibovitch et al., 1998). In a practical sense, it is fortunate that such individuals are relatively immobile, as otherwise they would certainly cause harm to themselves due to their decreased motor abilities and concomitant unawareness of deficits. Still, such individuals require close supervision, particularly as their motor deficits start to resolve. As motor recovery ensues, neglect also tends to diminish, again suggesting the prognostic value of early identification and assessment of neglect syndromes (Farne et al., 2004). Still, in two patients with similar levels of stroke severity, the one with neglect will likely have an overall poorer functional outcome (Buxbaum et al., 2004; Katz, Hartmann-Maeir, Ring, and Soroker, 1999).

Current thinking conceptualizes neglect as having numerous distinct subtypes. Deficits can be seen in the direction of attention to space that is within reaching distance of the individual or in failure to attend to extrapersonal space that falls outside of one's reach. Perhaps the most recognized deficit applies to personal neglect, whereby the individual fails to groom or dress one side of the body. In addition, neglect may take the form of failure to initiate or respond motorically in the affected hemispace, which can present similar to akinesia but be limited to one hemispace (Coslett, Bowers, and Heilman, 1987). Not surprisingly, the above authors have linked this variant of neglect to lesions of the frontal lobe, while more perceptual neglect tends to occur with more posterior damage, although controversy exists regarding the ability to localize subtypes of neglect to unique regions (Buxbaum et al., 2004). Lastly, the idea or "representation" of space may be lost, in that the individual may not be able to conceptualize of the affected hemispace. We are often able to replicate the classic paradigm first described by Bisiach and Luzzatti (1978) whereby patients have not been able to fully describe a common scene (e.g., facing the town square or city hall) as they neglect the affected hemispace but will correctly report such details (and omit their previous description) when asked to imagine that they are facing the other direction. Thus, it is not surprising that no unifying theory of neglect has emerged, given that visuospatial, sensory, motor, memory, and other "higher order" systems have been implicated in this deficit.

Such knowledge of the heterogeneity of the syndrome also guides the assessment of neglect. While seemingly one of the least quantifiable neurobehavioral syndromes, a variety of instruments have been created to test various for aspects of neglect (for an excellent review see Menon and Korner-Bitensky, 2004). In addition, this process is also conducted at the bedside with numerous nonstandardized instruments. Regardless, many instruments share

common components such as line bisection, cancellation of letters or shapes, free-hand drawings (e.g., clocks, flowers, houses, etc.) and copying of figures, or reading (e.g., the Indented Paragraph Test; Caplan, 1987). Additionally, tasks of mental imagery such as the one previously mentioned, tests of attention to body and extrapersonal space, geographical orientation, and observation in naturalistic settings all can yield clues in the diagnosis of neglect. Heilman, Watson, and Valenstein (1995) propose that testing for sensory inattention and extinction is done across three modalities: somesthetic, visual, and auditory. Testing for motor neglect includes observation in naturalistic settings, having the patient look both toward and away from ipsi- and contralateral stimuli (which, in our experience, is difficult due to many patient's instinct to move their head and/or trunk in relation to the stimulus), and having them identify (with their eyes closed) the midline of their body and of their immediate space. Perhaps the most popular method of testing consists of line bisection, cancellation tasks, and having the patient engage in spontaneous drawings to observe not only for neglect in the reproduced images but also in terms of placement on the page. All written tasks must be presented at the patient's midline and care must be taken to observe the patient's head and trunk movements to make sure that they are not trying to compensate for their neglect (although this is valuable information in its own right). Not surprisingly, given the heterogeneous nature of neglect, it has been suggested that a multimodal approach is necessary, as there is not a single test that is sensitive enough to capture all aspects of neglect (Maeshima et al., 2001).

Hence, it follows that numerous methods of treatment have been proposed. It has long been observed that right-sided strokes often carry with them the sequelae of decreased arousal. Thus, attempts have been made both pharmacologically (e.g., stimulants, dopamine agonists such as bromocriptine, etc.) and psychologically (teaching patients to self-alert) to improve arousal. While preliminary, some positive results have been described, although not consistently (for an excellent review of this and other treatments see Pierce and Buxbaum, 2002). Early attempts at improving visual scanning have shown short-term results but need both replication and proven ability to show generalizing results (e.g., Robertson, Gray, Pentland, and Waite, 1990). Modalities such as eye patching, prism glasses, and mental imagery training have also been reported to have some success, although, again, the literature is scant and in need of further replication (Pierce and Buxbaum, 2002). Perhaps one of the most interesting current lines of research has to do with hemispheric activation theories that propose that even small movements of the affected hand in the affected hemispace can result in decreased neglect. Such theories posit that such actions work to "reactivate" or recruit overlapping neural systems that are involved in the representation of hemispace (Robertson and North, 1993). Efforts have also begun to utilize a similar paradigm of motor activation for the recovery of language abilities after stroke (Raymer, Rowland, Haley, and Crosson, 2002). In addition, if such techniques prove worthwhile, they may be integrated into and complimented by the constraint-induced movement therapy techniques previously discussed (Taub and Wolf, 1997).

PERCEPTUAL DISORDERS

It is well recognized that visual and perceptual disorders can commonly occur after strokes. That being said, it is not unusual for these disorders to be missed in patients who have not undergone a neuropsychological examination. Unlike aphasia or neglect, whose presence is often glaringly obvious, visual-perceptual disorders, short of significant visual field cuts or frank neglect, can be quite subtle in terms of their expression. Their impact on outcome after stroke, however, is well established, especially in limiting the performance of activities of daily living (ADLs; Rubio, and Van Deusen, 1995; Titus, Gall, Yerxa, Roberson, and Mack, 1991). In fact, one of the most recognized ADL disorders, dressing apraxia, is being conceptualized as a sequelae of disturbed visuospatial abilities instead of an apraxia (Fitzgerald, McKelvey, and Szeligo, 2002). Some studies have shown that perceptual disorders have a greater impact than cognitive (e.g., language, memory, problem solving, etc.) difficulties on the level of disability seen acutely after stroke (Mercier, Audet, Hébert, Rochette, and Dubois, 2001). Thus, while often not the easiest of phenomena to explain to stroke patients and their families, perceptual disorders certainly need to be assessed and treated after stroke.

Perception often implies the creation of mental images in secondary and tertiary association cortices after initial processing in the visual cortex. The concept of a ventral (“what”) and a dorsal (“where”) stream comprising the outputs of visual cortex is well known (Mishkin, Ungerleider, and Macko 2000). Benton and Tranel (1993) conceptualized damage to these routes as contributing to three types of disorders: visuoperceptual, visuospatial, and visuoconstructive. Visuoperceptual disorders suggest impairments in processing forms, patterns, colors, shapes, and other features and could lead to visual object agnosia, prosopagnosia (defective facial recognition), and difficulties in color recognition. Visuospatial disorders consist of difficulties in localizing objects in space, defective judgments of direction and space, and defective topographical orientation. Deficits in either written drawing or copying of objects/shapes or in manual manipulation of objects to create or match models or figures comprises visuoconstructive deficits. While traditional logic suggests that the incidence of these disorders is closely associated with right or non-language dominant hemisphere damage, individuals with dominant hemisphere lesions also can show difficulties in some of the areas listed above. For example, Varney (2002) suggests that a significant proportion of individuals with reading disorders secondary to dominant hemisphere lesions often had visuoperceptive deficits that hindered them from recognizing letters and letter-like shapes.

Assessment of perceptual disorders follows from the theories posited above. In practice, the presence of hemiparesis, aphasia, neglect, and/or primary visual disturbances (e.g., field cuts) can often make the assessment of perception challenging. Benton, Sivian, Hamsher, Varney, and Spreen (1994) highlight many of the tests from Benton’s lab that have shown efficacy in assessing perceptual disorders, including measures of facial recognition, line orientation, visual form discrimination, and three-dimensional block construction. The Benton Visual Retention Test (Sivan, 1992), the Rey-Osterrieth Complex Figure Test (Rey, 1941), and the Block Design test from the WAIS-III (Wechsler, 1997) are also commonly used. Tests such as the Motor-Free Visual Perception Test (MVPT-III; Colarusso and Hammill, 2003) may also be utilized when hemiparesis or motor discoordination is present. As

previously stated, measures of perceptual abilities have been shown to be predictive of outcome after stroke rehabilitation and of post-discharge skills such as driving (Korner-Bitensky et al., 2000).

MEMORY DISORDERS

Perhaps the most frequent cognitive complaint that a rehabilitation neuropsychologist will hear from a stroke patient is that of reduced memory abilities. Formal testing may or may not find an actual memory disorder, per se, but rather difficulties in naming, attention, concentration, or organization of storage and/or recall as the cause for what is perceived as a memory difficulty. Still, in our experience, it is rare for a person to complain of difficulties in the components that underlie effective memory functioning, but rather notice the end result of poor retrieval from memory.

Much of our knowledge of memory functioning results from studies of classic cases of diseases that affect mesial temporal and diencephalic structures (e.g., Korsakoff's, herpes encephalitis, etc). Given that these conditions often affect these areas bilaterally, little has been written about strokes and these types of memory disorders simply because of the relative rarity of having two circumscribed strokes. Rather, the literature on stroke has primarily contributed to our understanding of memory disorders through the examination of frontal and thalamic systems.

Perhaps the most recognized amnesic disorder associated with the frontal lobes is the "ACoA syndrome" associated with anterior communicating artery aneurysm ruptures. This proposed syndrome features personality change (decreased awareness, disinhibition, etc.), impaired executive functioning, confabulation, and dense amnesia (DeLuca and Diamond, 1995). While other cognitive functions (i.e., visual-spatial, concentration, etc.), can be relatively spared, these individuals will show marked deficits in verbal recall of information (DeLuca, 1992). In addition, while impaired retrieval from memory is a hallmark of this disorder, some research suggests that impaired acquisition may be more affected than recall in a subgroup of patients with ruptured ACoA aneurysms (Diamond, Deluca, and Kelley, 1997).

The thalamus is one of the few subcortical structures that has received extensive investigation for its role in memory after stroke. Reports of verbal and visual-spatial memory deficits after thalamic infarcts, either in the dominant or non-dominant hemisphere, are plentiful in the literature (e.g., Kopelman et al., 2001). If such lesions are bilateral, frank amnesia is often seen and can be long lasting. It is not uncommon in such cases to see severe deficits in both recall and recognition of newly learned material along with some degree of retrograde amnesia (Isaac et al., 1998; Gentilini, DeRenzi, and Crisi, 1987). Interestingly, while the basal ganglia has been extensively researched in terms of its role in memory disorders with Parkinson's and Huntington's patients, little work has been forthcoming from the literature as to deficits following stroke (Crosson, 1992).

While strokes to the specific areas listed above can cause frank amnesic syndromes, the amount of general cognitive disorganization which accompanies most strokes cannot be underestimated and is often expressed through reduced memory. For example, Mast et al. (1999) found that approximately 35% of their older rehabilitation population that had experienced a stroke met criteria for dementia. Similarly, Ruchinskas, Repetz, and Singer

(2001) found that 70% of their older stroke population scored below a suggested cutting score for dementia on a cognitive screening instrument. What must be noted of all of this research, however, is that patients, even with severe memory disorders, can make functional or physical gains in rehabilitation. Many reasons for this phenomena have been suggested, but perhaps the most cogent explanation is that the areas that neuropsychologists traditionally focus on during their assessment (e.g., verbal and visual memory, attention, etc.) may not play as important an aspect in the physical rehabilitation arena as do other areas of cognition. For example, studies have hypothesized, suggested, and confirmed that procedural memory is dissociable from declarative memory and plays an integral part in the rehabilitation process (Ruchinskias et al., 2000; Patrick, Leber, and Johnston, 1996; Ewert, Levin, Watson, and Kalisky, 1989). This suggests that the neuropsychologist in the rehabilitation setting should incorporate a measure of procedural memory (such as the Rivermead Behavioral Memory Test; Wilson, Cockburn, and Baddeley, 1985), even at the bedside, in the initial evaluation, lest the treatment team become too concerned that the patient cannot verbalize recalling their exercises (yet can learn and perform them from day to day!).

EXECUTIVE FUNCTIONING

Executive functioning refers to a person's ability to plan, initiate, sequence, and monitor behavior that is complex and goal directed (Royall et al., 2002). Executive functioning includes the ability to formulate goals and recognize the long-term consequences of the action being considered. In addition, this area of functioning includes the ability to generate multiple alternative responses to a situation, choose between behavioral alternatives, self-monitor to determine if the behavior taken is accurate, and self-correct the behavioral response as needed. Additionally, the ability to focus and persist in the face of distraction, without becoming so focused that one loses the ability to determine the salience of new information, is a hallmark feature of the executive functions (Malloy, Cohen, and Jenkins, 1998). These functions are necessary for the individual to survive and thrive when managing his/her responses to the internal and external environment and are crucial for determining and engaging in socially appropriate behavior.

While executive functions have traditionally been seen as residing in the frontal lobes, one cannot downplay the numerous frontal-cortical and frontal-subcortical connections that also contribute to these skills. Cummings (1993) provides an excellent discussion of the frontal-subcortical circuits that are thought to form the substrate of the executive functions. Frontally based deficits fall in three realms. The traditional "executive deficits" (e.g., decreased abstraction, reasoning, and awareness) are typically associated with lesions in the dorsolateral prefrontal circuit. Deficits involving disinhibition are typically associated with lesions in the orbitofrontal circuit. Apathy is typically associated with lesions in the anterior cingulate circuit.

Damage to these underlying substrates and circuits leads to impairments in different areas of executive functioning. The executive function syndrome, involving damage to the dorsolateral prefrontal circuit, is characterized by deficits in initiating, planning, monitoring, and maintaining flexible behavior. Patients with this type deficit have problems integrating elements into a whole. In addition, behavioral responding is often characterized by a limited

response set with loss of set and/or perseveration. The disinhibition syndrome, arising from damage to the orbitofrontal circuit, is characterized by disinhibited social behavior. Personality change, poor inhibition, impulsivity and social inappropriateness are characteristic features. Confabulation and emotional lability are also often seen. There is often a significant change in affect with both insight and judgment impaired. The apathetic syndrome, due to lesions in the anterior cingulate circuit, is characterized by failure to respond to environmental stimulation. General apathy in spontaneous actions and motivation is characteristic (Malloy et al., 1998).

Vataja et al. (2003) discusses the MRI correlates of executive dysfunction specifically in stroke patients. In their sample of stroke patients, 34% exhibited problems with executive function. The patients with executive dysfunction had a greater frequency of brain infarctions overall and in the left hemisphere. In addition to this finding, lesions in frontal-subcortical circuits were more common in patients with deficits in executive function. Some patients with pontine lesions also demonstrated executive dysfunction, but this and similar studies' findings may have been due to more extensive ischemic changes in these patients overall (Ruchinskas, 1998). The conclusion from this neuroradiological study was that brain infarcts and lesions of the white matter, which affect frontal-subcortical circuits, create an increased risk for the development of deficits of executive functioning in stroke patients.

While the idea of unawareness of deficits after stroke is well recognized, the decreased recognition of limitations is by no means solely restricted to this population. In fact, the difficulties of correctly defining what is "organically" based unawareness versus "psychologically" based denial are encountered on a daily basis in a rehabilitation setting. Perhaps the most accommodating viewpoint sees awareness as a continuum from frank denial and unawareness to that of almost neurotically based over-focus on perceived deficits that can occur in those with and without neurological disease. While too large to cover in this work, the reader is encouraged to consult Prigatano and Schacter (1991) and Prigatano's subsequent works for a coherent review of this subject. That said, we will briefly review unawareness syndromes that accompany stroke. Historically, it was thought that psychological denial was responsible for much lack of awareness following neurological injury. We now understand that anosognosia, or lack of awareness due to cortical damage, plays a major role in the development of lack of awareness in patients with neurological injury including stroke. The term "anosognosia" was coined by Babinski in 1914 and is considered to be an impairment in executive functioning (Hibbard, Gordon, and Stein, 1992). Traditionally, unawareness of deficit has been believed to be more common following damage to the right hemisphere, especially following basal ganglia and parietal damage, but aspects of unawareness can be seen following damage to the left hemisphere or most any cortical area (Prigatano and Schacter, 1991).

Primary to the belief of decreased awareness after stroke is that patients would consistently overestimate their physical and mental abilities and that this phenomena would occur more often in individuals with right hemisphere strokes. This hypothesis has not been consistently supported. For example, Hibbard et al. (1992) found that the majority of patients with both right and left-hemisphere damage were accurate in rating the extent of the physical impairments they experienced following stroke, although both groups demonstrated an overall lack of accuracy when rating the mood, language, and cognitive changes resulting from stroke. In contrast, two recent studies found that stroke patients rated themselves as less impaired in motor abilities than they were rated by rehabilitation staff although they rated

themselves as more impaired in cognitive and emotional aspects than staff rated them. (Fisher, Trexler, and Gauggel 2004; Gauggel, Peleska, and Bode, 2000).

Regardless, measures of executive function have shown a relationship with ultimate rehabilitation outcome. Tests of executive functioning have been shown to be predictive of ultimate ADL and instrumental ADL abilities (Cahn-Weiner, Boyle, and Malloy, 2002; LaBuda and Lichtenberg, 1999). Thus, it can be hypothesized that high-level cognitive functions are necessary for ultimate rehabilitation success once the patient's sensory and physical impairments have resolved. An alternative possibility is that high-level cognitive functions allow the patient to compensate for unresolved impairments in the other domains. At any rate, while inpatient rehabilitation places a high priority on physical and motor functioning, the assessment and treatment of executive functions cannot be downplayed in respect to the ultimate success or failure in rehabilitation (Hanks, Rapport, Millis, and Deshpande, 1999).

There are several strategies that can be implemented in the assessment of the executive functions. The reader is encouraged to consult Royall et al. (2002) for an excellent review of the overall concepts of executive functioning, the specific executive control circuits and systems, and assessment of executive functions using both bedside and more formal neuropsychological measures. Included in that discussion are the Behavioral Dyscontrol Scale, the Executive Interview, the executive clock drawing task, and the Frontal Assessment Battery.

Malloy et al. (1998) also provide a very nice review of specific assessment approaches. They suggest that in the assessment of the primary motor area the neuropsychologist will find it helpful to perform some aspects of a neurological-type exam, such as having the patient squeeze your fingers. In addition, they suggest using the Grip Strength/Hand Dynamometer and Finger Tapping Test when assessing this area. To assess the premotor area, the Purdue Pegboard Test or Grooved Pegboard Test can be helpful. The frontal eye fields can be productively assessed by using visual search tasks. The dorsolateral area can be assessed by using measures of word fluency, figural fluency, and the Wisconsin Card Sorting Test. The orbital and basal areas can be assessed by using a smell identification test, go/no go tasks and/or the Stroop test. For an extensive review of other measures that can be used to assess the various elements of executive functioning in the assessment of post-acute patients, please see Lezak et al. (2004).

Deficits in executive function may be, but are not necessarily, grossly evident during the rehabilitation process. In the highly structured atmosphere of rehabilitation, difficulties in executive functioning may be relatively subtle and easily missed by the treatment team. It is helpful for the neuropsychologist to be attuned to the concepts and impairment possibilities discussed above so that he/she can recognize the presence of these deficits and the potential impact on the patient's ability to achieve maximum recovery following stroke. The neuropsychologist can then work with the patient, patient's family, and other rehabilitation professionals in identifying, understanding, and addressing these issues so the patient is given the best opportunity to achieve the maximum benefit from the rehabilitation program. While the concept of executive functions may be difficult to explain to individuals and families, there are some simple experiments that can be utilized to illuminate this point. For example, we often ask patients to find their way to the hospital cafeteria, buy an item with the money that we provide, and return within a certain time period (usually with a staff member accompanying but not aiding them). This seemingly simple everyday task can show deficits

in planning, organization, time sense, and other executive functions that will be glaringly obvious to the patient (sometimes) and to the family. At times, the results of these experiments also surprise staff that believe the patient to be functioning at a high level. It is often necessary to mention the nature of inpatient rehabilitation as one of a highly structured existence. Taking away external structure and forcing the patient to use their internal mechanisms often reveals weakness in executive functions and can be a guide as to how much supervision will be necessary upon discharge. Improvements in ADLs can be gained by targeting executive functioning during the rehabilitation process (Honda, 1999). Such strategies usually focus on remediation of deficits or the use of external compensation strategies and devices (i.e., pagers, notebooks, etc.), which are often times the most efficacious approaches when difficulties in awareness or monitoring are present. The reader is encouraged to consult sources such as Sohlberg and Mateer (2001) for a comprehensive approach to remediating executive dysfunction.

CONCLUSION: PRACTICAL ASPECTS OF NEUROPSYCHOLOGY IN STROKE REHABILITATION

The practice of neuropsychology in an acute or post-acute stroke rehabilitation environment is a challenging yet rewarding venture. In acute settings, the practice involves equal parts behavioral neurology and neuropsychology in that the first aspect of the neuropsychological evaluation involves determining the neurobehavioral syndromes that are present that can affect formal neuropsychological assessment. Included in this process is an evaluation of the nature and degree of hemiparesis, ataxia, and motor incoordination. Sensory systems such as vision, audition, and cutaneous sensation must be assessed. Comprehension must be assured, and the ability to express oneself across many mediums must be evaluated. Upon completion of this initial evaluation, the clinician should have a good understanding of the degree of motor weakness/incoordination, sensory deficits (e.g., field cuts, decreased hearing, etc.), aphasia, dysarthria, neglect, etc., that can potentially affect further evaluation (and the rehabilitation process as a whole).

After deciding which cognitive instruments to use, the clinician still faces numerous challenges. Perhaps the most common, yet largely ignored, factor facing the clinician is that of post-stroke fatigue. During the course of the day, an individual is expected to participate in multiple therapies that consume a large portion of their waking hours. During that time, trying to catch an individual while they are “fresh” enough to perform tests that are not always interesting and may challenge areas of cognitive deficit can be a significant challenge. Post-stroke fatigue is quite common (Glader, Stegmayr, and Asplund, 2002) and may also explain some of the performance variability, both in cognitive and physical domains, which is observed by the treatment team. Additionally, there is research that suggests that post-stroke fatigue may not ease during the rehabilitation stay and may, in fact, be quite long-lasting (De Groot, Phillips, and Eskes, 2003). As previously mentioned, difficulties in arousal may also be quite prevalent in (particularly right-sided) strokes, and pose similar problems for assessment and treatment. Lastly, as noted at the beginning of this chapter, the existence of post-stroke depression can negatively impact the evaluation process as well as progress in the

rehabilitation program as a whole in terms of making it difficult for the patient to put forward his best cooperation or effort.

The neuropsychologist working with stroke patients in the rehabilitation setting needs a skill set that is both broad and deep in order to function optimally. The neuropsychologist must be well-versed in neurological syndromes and issues, neurocognitive functioning, neuropsychological assessment, and the assessment and treatment of psychological/emotional issues following stroke. The neuropsychologist in this setting must adopt a focused and practical philosophy of assessment in order to provide the most crucial information to the patient, family, and other team members in a timely fashion. In addition, interventions in this environment to address cognitive and/or emotional issues must be pragmatic and must be undertaken quickly. Besides a large technical knowledge base required to perform successfully as a neuropsychologist in this setting, one must also be attuned to the multiple formal and informal power structures in the rehabilitation environment as well as to the interpersonal and interprofessional relationships within this setting. The neuropsychologist must develop significant diplomacy skills when working with the multiple other treating professionals in this environment. Unlike some practice settings in which the neuropsychologist is the sole provider of services, the neuropsychologist in the rehabilitation setting must be comfortable interacting with multiple other professionals. Blair and Gorman (2003) offer several "survival tips" to assist the neuropsychologist in understanding the social complexities of the rehabilitation setting, adapting neuropsychology practice to this setting, and making the optimal adjustment to this clinical milieu. The neuropsychologist who learns to function effectively in this setting has much to offer the patients, families, and other clinicians and indeed makes a unique and valuable contribution to the rehabilitation and healing of patients following stroke.

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Chapter 46

**EFFECTS OF WALKING SPEEDS
AND NORMALIZATION METHODS
ON THE MUSCULAR ACTIVITY OF THE PARETIC
LOWER LIMB IN STROKE SUBJECTS**

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ABSTRACT

The purpose of this chapter was to compare the magnitude of the electromyographic (EMG) signals of the paretic lower limb muscles of stroke subjects between comfortable and maximal walking speeds, considering two methods of normalization. A convenience sample of 10 chronic stroke subjects walked at their self-selected comfortable and maximal walking speeds. The EMG signals were collected from the rectus femoris, biceps femoris, tibialis anterior, and lateral gastrocnemius muscles of the paretic lower limb and were quantified by the root mean squares and normalized by the mean and peak dynamic methods. For all muscles, the ANOVA performed on the mean EMG values over specific periods of muscular activity revealed significant interactions between walking speeds and methods of normalization ($9.02 < F < 31.24$; $0.001 < p < 0.016$). The effects of the methods were greatest at the maximal speeds. The inter-subject variability was also modified by the normalization method, but not consistently for all of the muscles, except for the rectus femoris. In general, the greatest inter-subject variability

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was observed at maximal walking speeds. The normalization methods and the walking speeds modified the EMG levels and the inter-subject variability. These findings should be considered when the impact of locomotor interventions on EMG values with stroke subjects is studied.

Keywords: Electromyography, stroke, gait analysis, walking speed.

INTRODUCTION

Stroke is one of the most common causes of long-term disability worldwide, and is an important public health concern (Geyh *et al.*, 2004). Among all disabilities demonstrated by stroke subjects, impaired walking is considered to be one of the most important (Geyh *et al.*, 2004; Olney and Richards, 1996). It is well recognized that changes in gait performance are the greatest contributors to functional disabilities post-stroke (Jorgensen *et al.*, 1995). In addition, the degree to which walking is impaired following a stroke relates to the severity of the subjects' lower motor extremity deficiencies (Perry *et al.*, 1995) and to the degree to which community subjects are functionally independent (Wade *et al.*, 1987). Furthermore, walking is the activity which stroke subjects rate as being the most important (Bohannon *et al.*, 1988). Considering these factors, several studies have provided better comprehension of gait disturbances of stroke subjects (Olney and Richards, 1996; Jorgensen *et al.*, 1995; Perry *et al.*, 1995; Wade *et al.*, 1987; Bohannon *et al.*, 1988; Bohannon, 1992; Nadeau *et al.*, 1999; Olney *et al.*, 1994; Salbach *et al.*, 2001; Teixeira-Salmela *et al.*, 2001).

One of the most employed variables to investigate gait performance is walking speed (Olney and Richards, 1996; Bohannon, 1992; Nadeau *et al.*, 1999; Olney *et al.*, 1994; Salbach *et al.*, 2001), given its objectivity (Bohannon, 1992; Salbach *et al.*, 2001) and importance for safe ambulation (Cohen *et al.*, 1987). Another variable often used in investigations regarding gait performance is the electromyographic (EMG) activity of the lower limb muscles (Burden *et al.*, 2003; Hesse *et al.*, 2001; Olney and Richards, 1996; Yang and Winter, 1984), since the most significant impairment for gait performance is the inability to generate voluntary muscle contractions of normal magnitudes and in a coordinated manner (Olney and Richards, 1996). However, there were no studies found which compared EMG activity of the lower limb muscles between comfortable and maximal walking speeds with stroke subjects.

Another important question which has not yet been well addressed is related to the optimal normalization method of the EMG signals to be applied in gait analyses of the paretic lower limb of stroke subjects. Burden *et al.* (2003) compared four different methods of normalization of the EMG signals recorded during gait of healthy subjects, walking on a treadmill at normal speeds. Despite their important results, some questions regarding the normalization still remain. One is related to the best normalization method to analyse the muscular activity of stroke subjects during gait. This is mainly for the muscles of the paretic lower limb, which showed decreased capacity to activate motor units, as well as reduced number of functioning motor units and motor unit firing rates (Carr and Shepherd, 1998; McComas *et al.*, 1973). Unanimous agreement on EMG of the lower limb of stroke subjects is the high levels of inter-individual variability (Den Otter *et al.*, 2007; Olney and Richards,

1996). One way of addressing this problem is the investigation of the normalization methods most suitable for the reduction of variability associated with other factors in gait patterns. Amongst the four methods of normalization which have been most investigated, two have been shown to reduce inter-individual variability: The mean and the peak dynamic methods (Burden *et al.*, 2003; Yang and Winter, 1984). Both methods have some advantages, since the normalized values are derived from walking trials (Yang and Winter, 1984).

The first aim of this chapter was to compare the magnitudes of the EMG signals of the paretic lower limb muscles of stroke subjects between comfortable and maximal walking speeds, considering two means of normalization, the mean and peak dynamic methods. The second was to describe the intra- and inter-subject variability of each method at both walking speeds. All comparisons were carried-out considering four different muscles of the paretic lower limb: The rectus femoris, lateral hamstrings (biceps femoris), tibialis anterior, and lateral gastrocnemius. At a given speed, for each muscle, the period of the gait cycle which showed the most important EMG levels, determined from the averaged EMG profiles, were considered for analyses.

METHODS

Subjects

Subjects with hemiparesis due to stroke were recruited from a rehabilitation center in the Montréal area (Institut de réadaptation Gingras-Lindsay-de-Montréal). Participants required the following criteria to participate: 1) had a chronic (six months or more) unilateral stroke, 2) were able to walk 10 meters independently with or without a cane, and 3) had residual weaknesses of the paretic lower limb. The following exclusion criteria were applied: 1) use of orthoses, 2) comprehensive aphasia, 3) incontinence, 4) unstable medical conditions, 5) histories of injuries, and 6) anesthesia of the lower limbs. This information was gathered on a clinical chart, from the participants themselves, or by their proxy. Authorization by the primary care physician was obtained, if necessary. All potential participants completed an informed and written consent prior to the assessment sessions in accordance with the ethical standards of the University ethical committee.

EVALUATION

Clinical Evaluations

For characterization purposes, demographic and clinical data were collected on all subjects by a trained physical therapist. Clinical data were related to the physical impairments of the lower limb evaluated by the lower extremity component (leg and foot) of the Chedoke-McMaster Stroke Assessment (Gowland *et al.*, 1993), balance (Berg Balance Scale) (Berg *et al.*, 1995), and by clinical measures of comfortable and maximal walking speeds, quantified by the five meter walking test (Salbach *et al.*, 2001).

Gait Evaluations

Gait parameters were collected during the five gait cycles at both self-selected comfortable and maximal speeds. Unilateral surface EMG signals of the following four selected muscles of the paretic lower limb were recorded during gait: The rectus femoris, biceps femoris, tibialis anterior, and lateral gastrocnemius. These muscles are recognized as the most important during gait (Olney and Richards, 1996; Winter, 1991) in studies with stroke subjects (Den Otter *et al.*, 2007; Engardt *et al.*, 1995).

Small, disposable, disc silver–silver chloride (Ag/AgCl) surface electrodes with a 13.2 mm² active surface area (AMBU_ Blue Sensor M), were arranged in a bipolar configuration with a two cm inter-electrode distance, and directly applied over the muscle bellies, parallel to their fiber orientation. A reference electrode was placed over the head of the fibula. Each electrode placement site was prepared using recommended preparation techniques (Cram *et al.*, 1998). The EMG cables were connected to a Noraxon Telemetry 900 portable integrated EMG multi-channel transmitter (Noraxon, Scottsdale, AZ) (weight=0.453 kg per transmitter), which was attached around the subjects' waist. All myoelectric signals underwent an analog-digital conversion before being relayed to the receiver/amplifier units. At this point, the myoelectrical signals were amplified with an overall gain of 2,000 and band-pass filtered (10–500 Hz). Finally, the signals were digitized at a sampling frequency of 1,200 Hz and stored on a computer using a custom-made Labview program (National Instruments, Austin, TX).

Electromyographic Data Processing

First, the EMG signals of all the collected gait trials were full wave rectified and band-pass filtered. All raw EMG signals were visually inspected following signal offset removal. Then, the stride characteristics of each gait cycle were determined considering the peak signals provided by the footswitches located on the soles of the shoes at the heel, metatarsal heads, and mid-foot. The EMG data were then, time normalized to 100% of the gait cycle using a two-point method. The EMG and the footswitch signals were processed by a locally developed software.

Out of five collected gait trials, the three which showed the most similar values for speed, cadence, and EMG activity were averaged and selected for analyses. The normalization process of the EMG signals was performed using the mean and the peak dynamic methods. For the mean dynamic method, the mean RMS values for the complete stride of the averaged trials were calculated for each participant. For the peak dynamic method, the maximal RMS values were calculated. To allow comparisons between the magnitudes of muscular activity at the comfortable and maximal walking speeds, the EMG signals at both speeds were normalized using the mean and peak values of the comfortable walking speed.

Finally, for each method and speed, the periods of the gait cycle where the EMG activity was the most important were visually identified for each muscle by careful observations and analyses of the EMG profiles (Figures 1 and 2), considering the specific phases of the gait cycle, as performed in previous studies (Den Otter *et al.*, 2004; Murray *et al.*, 1984; Shiavi *et al.*, 1987; Winter, 1991).

Over each of the selected periods, the means of the EMG values obtained by each normalization method was calculated. Then, the ratio of the differences between the mean

values of the two methods over the peak dynamic method, were calculated to better understand the amplitude differences between these normalization methods. The same approach was used to assess the differences between gait speeds for a given normalization method.

Statistical Analyses

Descriptive statistics (means and standard deviations) were computed for all demographic, clinical, and EMG variables and tests for normality were carried out for all investigated parameters. Two-way repeated measure ANOVAs were employed to investigate the mean and interaction effects between the walking speeds (comfortable and maximal) and the normalization methods (mean and peak).

The coefficients of variation (CV), obtained by the ratio of the standard deviations to the mean values, expressed in percentages (Portney and Watkins, 2000), were used to determine the intra-subject variability for each walking speed, as well as the inter-individual variability for each method of normalization at each walking speed. All statistical analyses were performed with the SPSS® software (version 15.0) with the significance level of $\alpha < 0.05$.

RESULTS

Subjects' Characteristics

Ten hemiparetic subjects, six men and four women, with a mean age of 60 ± 12.4 years (ranging from 46 to 78 years) and a mean time post-stroke of 62.5 ± 52.8 months (ranging from 25 to 172 months), participated. Five subjects were right side paretic. The median Chedoke scores were five and four for the leg and foot, respectively, and the Berg Balance Scale score was 53/56. During the five meter walking test, the mean comfortable walking speed was 0.82 ± 0.29 m/s (ranging from 0.41 to 1.44) and the mean maximal walking speed was 1.22 ± 0.47 m/s (ranging from 0.42 to 2.03). All subjects were able to increase their maximal walking speeds, when compared to the comfortable ones ($p \leq 0.001$).

During EMG data collection, the mean comfortable speed was 0.69 ± 0.30 m/s (ranging from 0.36 to 1.39) and the mean maximal walking was 1.19 ± 0.44 m/s (ranging from 0.49 to 1.71). Once again, all subjects were able to increase their maximal walking speeds, when compared to their comfortable ones ($p \leq 0.001$).

Pattern Characteristics of the EMG Activity

As can be observed in Figures 1 and 2, for all muscles, the average pattern characteristics of the EMG activity were similar between the two methods of normalization, although the EMG levels and the inter-subject variability (one standard deviation) were greatest for the data normalized by the mean dynamic method. The following descriptions regarding the EMG profiles of each muscle could thus, be applied to both methods of normalization.

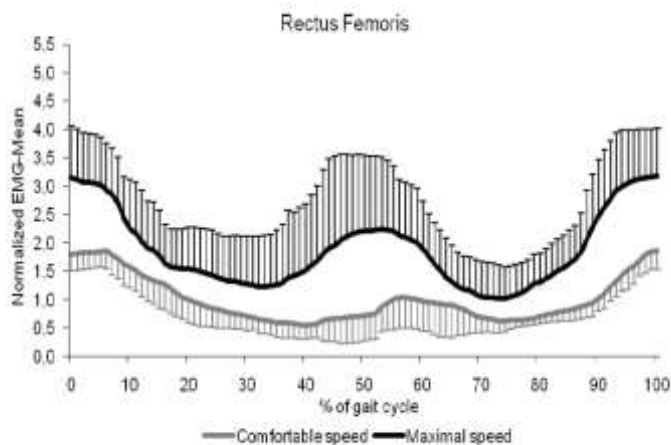
The patterns of the rectus femoris muscle were biphasic at both walking speeds. Considering the mean profiles, the first period of EMG activity during the gait cycle began at mid-swing (~80%) and finished before mid-stance, 20 and 30% for the maximal and comfortable speeds, respectively. The second period of activity was clearer at maximal speeds and varied from the second half of the stance to the initial swing (maximal speed: 35%-60%; comfortable speed: 50-70%). The three periods of EMG activity were selected for analyses: 0-20%, 35-70%, and 80-100% of the gait cycle. During these periods, the maximal values of the normalized EMG activities were observed just prior to the initial contact.

The patterns of the biceps femoris were monophasic at both walking speeds. The period of the gait cycle of major EMG activity began at mid-swing (~75%), continued until the end of the gait cycle, and ended at the terminal stance (~35%). The highest EMG amplitudes were depicted just prior to the initial contact. For this specific muscle, two periods of EMG activity were selected for analyses: 0-35% and 75-100%.

The tibialis anterior showed biphasic patterns at both walking speeds. The first period of EMG activity began at mid-swing (~80%) and continued until mid-stance (~15%). The second period was observed at the terminal stance and continued until the initial swing (comfortable speed: 45-70%; maximal speed: 40-60%). Three periods were then, selected for analyses: 0-15%, 40-70%, and 80-100%. The maximal levels of EMG activity were often observed at the initial contact. It should also be noted that this muscle had the most similar EMG patterns and amplitudes between the walking speeds (Figures 1 and 2).

The lateral gastrocnemius demonstrated distinct patterns at each speed. At the comfortable speed, monophasic patterns of EMG activity were observed from mid-stance (~25%) to the pre-swing (55%).

The highest EMG values were seen in the middle of the terminal stance. At the maximal speed, biphasic patterns were observed: One period began at the terminal swing (~85%) and continued until mid-stance (~20%), and the second period was from around 25% to 55% of stance. Since this period was common for both speeds, it was thus, selected for analyses.



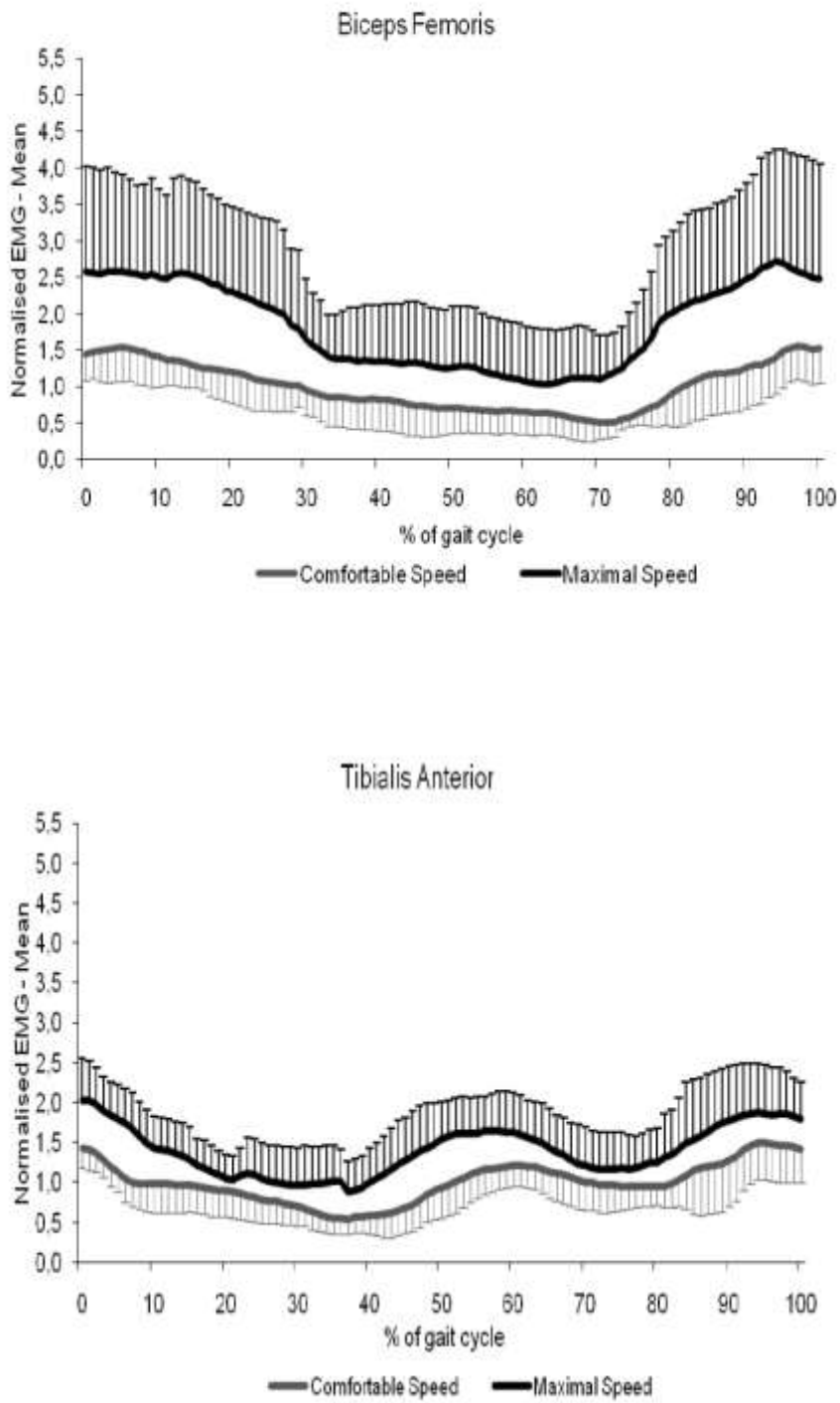


Figure 1. (Continued)

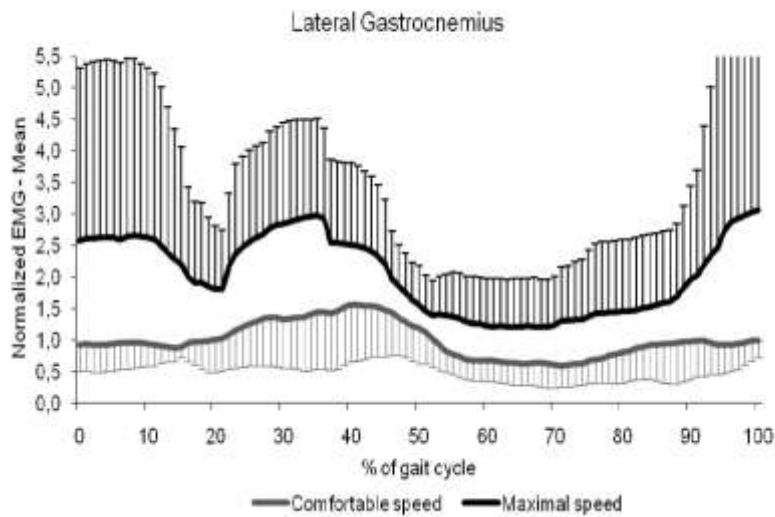


Figure 1. Electromyographic activity (means $\pm$ SD) normalized by the mean dynamic method during one complete gait cycle of the paretic lower limb muscles, both at comfortable and maximal speeds.

Comparisons Between the Comfortable and Maximal Walking Speeds Considering the Methods of Normalization

The magnitudes of the EMG activity at maximal walking speeds were always greater than those at comfortable speeds for both methods of normalization (Figure 3). For the mean method, the ratios provided in Table 1, ranged from 32 to 126%, greater at the maximal speed. Corresponding values for the peak method ranged from 34 to 122%.

For all statistical analyses, the ANOVAs revealed interaction effects between walking speeds and the methods of normalization ($9.02 < F < 31.24$; $0.001 < p < 0.016$), indicating that the normalization method had greater effects on the mean data at the maximal speed with the steepest decreasing slope (Figure 3).

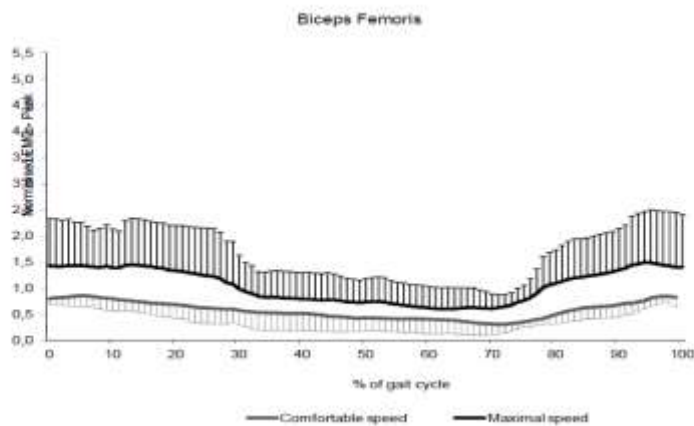
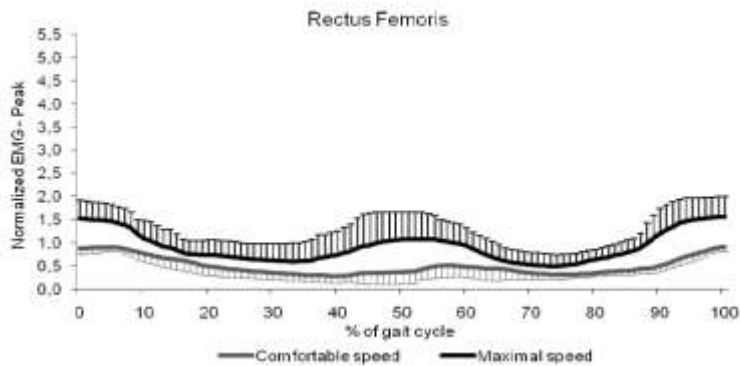
Consequently, for most muscles over the analyzed periods of the gait cycles, the greatest differences between speeds were observed for the data normalized by the mean method (Figure 3).

As given in Table 1, the ratios computed from the mean values reported in Figure 3, revealed that at the comfortable and maximal walking speeds, the values of the mean method of normalization were respectively 64 to 100% and 67 to 103% greater than the values obtained by the peak method.

Table 1. Mean magnitudes of the electromyographic activity ratios (%) between each method of normalization at comfortable and maximal walking speeds and between walking speeds normalized by the peak and mean dynamic methods.

Muscle	Gait Cycle	Comfortable: Mean to peak	Maximal: Mean to peak	Peak method Maximal to comfortable	Mean method Maximal to comfortable
RF	0 to 20%	100.00	102.61	57.53	59.59
	35 to 70%	100.00	103.30	121.95	125.61
	80 to 100%	98.31	101.68	101.69	105.13
BF	0 to 35%	75.71	74.80	81.43	80.49
	75 to 100%	82.81	81.60	95.31	94.02
TA	0 to 15%	64.18	67.35	46.27	49.09
	40 to 70%	66.10	68.24	44.07	45.92
	80 to 100%	74.32	71.72	33.78	31.78
LG	25 to 55%	116.39	109.01	81.97	75.76

RF: Rectus femoris; BF: Biceps femoris; TA: Tibialis anterior; LG: Lateral gastrocnemius.



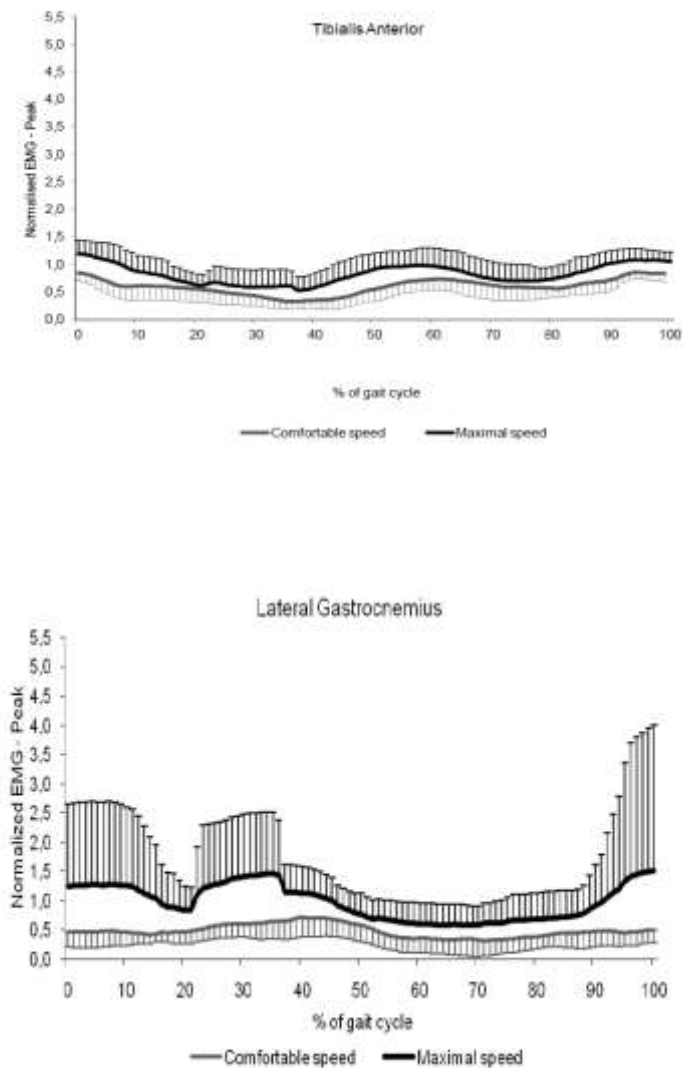


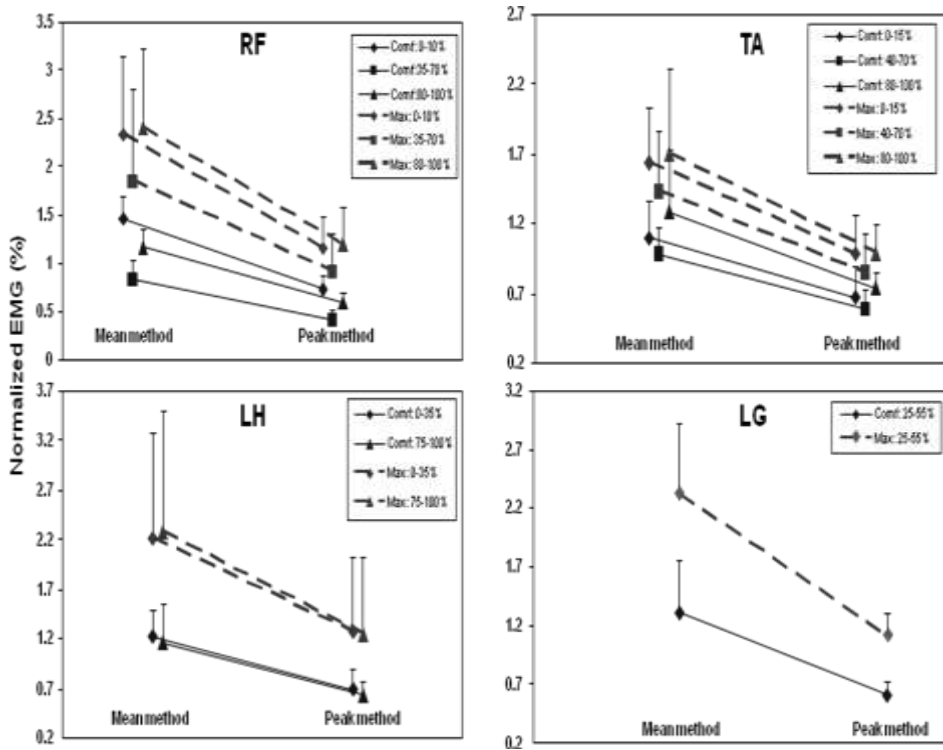
Figure 2. Electromyographic activity (means $\pm$ SD) normalized by the peak dynamic method during one complete gait cycle of the paretic lower limb muscles, both at comfortable and maximal speeds.

Intra- and Inter-Subject Variability of the EMG Signal Amplitudes

Table 2 shows the range of the coefficients of variation related to the intra-subject variability at both walking speeds and normalization methods. For both speeds and methods of normalization, the maximal values were lower than 20%, which indicated that the intra-subject variability was low.

As shown in Figures 1 and 2, differences existed regarding the inter-subject variability of the data obtained by the two normalization methods, with greater standard deviation values

generally associated with the mean method. Observations of the coefficients of variation (Table 3) did not reveal consistent effects of the method on the inter-subject relative variability, except for the rectus femoris, which demonstrated no method effects, since for all analysed gait periods, the coefficient of variation values, obtained by both normalization methods, were lower than 10%.



RF: Rectus Femoris; BF: Biceps Femoris; TA: Tibialis Anterior; LG: Lateral Gastrocnemius; Comf: Comfortable speed; Max: Maximal speed.

Figure 3. Mean EMG ($\pm$ SD) computed over specific periods normalized by both methods at comfortable and maximal speeds.

Table 2. Range of intra-subject variability (coefficients of variation, %) of the electromyographic activity at both speeds and normalization methods.

Muscle	Gait cycle	Mean method		Peak method	
		Comfortable speed	Maximal speed	Comfortable speed	Maximal speed
RF	0 to 20%	3.23-11.20	3.24-11.43	3.02-16.66	3.73-12.53
	35 to 70%	2.31-19.74	3.85-18.80	6.13-12.14	4.83-19.10
	80 to 100%	5.83-13.70	2.87-13.98	4.50-14.05	3.11-13.29
BF	0 to 35%	0.17-9.17	2.34-19.23	0.40-8.63	1.78-13.55
	75 to 100%	3.63-13.77	3.36-17.30	4.58-14.72	3.15-15.20
TA	0 to 15%	1.27-15.37	1.57-13.83	2.67-11.76	2.42-14.34
	40 to 70%	3.50-8.27	2.44-13.88	3.87-11.62	2.47-19.37
	80 to 100%	4.20-12.80	0.63-10.60	2.34-13.79	1.41-15.74
LG	25 to 55%	4.73-18.07	1.45-13.57	5.76-13.57	3.08-16.44

RF: Rectus femoris; BF: Biceps femoris; TA: Tibialis anterior; LG: Lateral gastrocnemius.

Table 3. Inter-subject variability (coefficients of variation, %) of the electromyographic activity at both speeds and normalization methods.

Muscle	Gait cycle	Mean method		Peak method	
		Comfortable speed	Maximal speed	Comfortable speed	Maximal Speed
RF	0 to 20%	15.07	34.76	17.81	28.70
	35 to 70%	24.39	50.81	24.39	43.96
	80 to 100%	14.53	34.17	15.25	32.77
BF	0 to 35%	20.33	47.75	28.57	59.84
	75 to 100%	33.33	54.19	20.31	60.80
TA	0 to 15%	23.64	23.78	32.84	28.57
	40 to 70%	19.39	30.07	23.73	32.94
	80 to 100%	34.11	35.88	14.86	20.20
LG	25 to 55%	40.15	36.64	34.43	45.95

RF: Rectus femoris; BF: Biceps femoris; TA: Tibialis anterior; LG: Lateral gastrocnemius.

Observations of the standard deviations (Figure 3) and the coefficients of variation (Table 3) showed that for most muscles over the analyzed periods of the gait cycle, the inter-subject variability of the EMG values at maximal speeds was greater than those obtained at comfortable walking speeds, independent of the method of normalization.

DISCUSSION

The overall aim of this chapter was to compare the magnitudes of the EMG signals of the paretic lower limb muscles of stroke subjects walking at comfortable and maximal walking speeds with two forms of normalization, the mean and the peak dynamic methods. The stroke participants had lower comfortable and maximal walking speeds, compared to values reported for healthy subjects. As reported by Shavi *et al.* (1987), able-bodied individuals showed a mean comfortable walking speed of 1.44 and maximum of 1.81 m/s. For the present stroke subjects, corresponding values were 0.69 and 1.19, which represented a mean reduction of 52.1 and 34.5%, respectively. Based upon the classification of walking handicaps in a stroke population by Perry *et al.* (1995), the participants fell within the category of “community-walkers” showing full independence in all home and community activities with low risks of falling. The present findings indicated that the method of normalization and the walking speeds modified the EMG activity on the paretic lower limb muscles. The patterns of the peak and the mean methods were differently affected by the walking speeds, since interactions between the walking speeds and normalization methods were observed for all periods of activities for all analyzed muscles.

Pattern Characteristics of EMG Activity

The patterns of the EMG activity were quite similar between the methods of normalization. The periods of gait cycles selected for analyses, which were related to the most important EMG levels of the averaged profiles, were almost equivalent to those chosen by Winter (1991) as being the most important peak activity levels observed during gait of

healthy subjects. However, some periods of the most relevant EMG levels of the stroke subjects were slightly longer than those described by Winter (1991), probably due to the fact that they walk slower than healthy subjects. As pointed out by den Otter *et al.* (2004), the amplitude of muscular activity increases with walking speed, due to the need for larger muscular force output. This is because the main role of the muscles in the control of walking speed is to accelerate and decelerate forces of body segments to allow safe forward progression (Winter, 1991). However, since visual inspection was used to determine the EMG onsets and offsets, it is possible that the differences could be partially attributed to the subjectivity of the method itself.

Individual analyses of the EMG activity of each muscle showed that for the rectus femoris and tibialis anterior muscles, the biphasic patterns of EMG activity were quite similar to those of healthy subjects at both walking speeds. As Winter (1991) described, for the rectus femoris, a peak of EMG activity was observed from the end to the beginning of the gait cycle, during weight acceptance phase and after the toe-off to pull the swinging limb forward. For the tibialis anterior, a peak in activity at the beginning of the gait cycle was observed, which corresponded to force generation in opposition to the plantar flexor ground reaction forces. A second important peak in the activity of the tibialis anterior was observed at the end of the stance and during the swing phases, which corresponded to the generation of force for foot clearance. In addition, over all of the analyzed muscles, the tibialis anterior showed the most consistent patterns between the comfortable and maximal walking speeds. For the biceps femoris, a peak in the EMG activity was also observed at the end and beginning of the gait cycle to prepare for weight acceptance.

Finally, for the lateral gastrocnemius, the monophasic patterns observed during comfortable walking speeds changed to biphasic ones at maximal speeds. A new period of EMG activity began at the terminal swing and continued until mid-stance. The period which was similar at both walking speeds was observed during the mid- and late-stance, which was related to the active plantar flexion of the foot and the generation of the most important impulses of energy (Winter, 1991). The new burst of EMG activity observed for this muscle from the terminal swing until mid-stance at maximal speeds might be related to the biomechanical demands associated to increases in walking speed (Shiavi *et al.*, 1987) and also to the spasticity of the plantar flexors. The lateral gastrocnemius can act both at the knee and ankle joints and during weight acceptance. The amount of energy absorption at the ankle and knee joints increases at higher walking speeds (Teixeira-Salmela *et al.*, 2008), which could explain the new period of EMG activity observed in this muscle from the terminal swing to mid-stance. In addition, it is important to point out that variance in the EMG signal at maximal speeds also seemed to increase during this period. Considering that this new burst of EMG activity could be related to the biomechanical demands associated with increases in walking speed (Shiavi *et al.*, 1987) and also to the spasticity of the plantar flexors, more motor units need to be recruited. Since the muscles of the paretic lower limb of subjects with stroke demonstrated decreased capacities to activate motor units, as well as reduced numbers of functioning motor units and the motor unit firing rates (Carr and Shepherd, 1998; McComas *et al.*, 1973), greater variability in the EMG signal is observed when more motor units were recruited (Nandedkar, 2002; Grosse *et al.*, 2002).

These results were also, in general, similar to those reported by Shiavi *et al.* (1987), who described the EMG activity pattern characteristics of the lower limb muscles at different walking speeds (free, fast, slow, and very slow) with healthy subjects. The rectus femoris and

tibialis anterior muscles showed biphasic patterns for all speeds, and a second distinct period in EMG activity of the gastrocnemius was developed at fast speeds during the swing-to-stance transition. Furthermore, the tibialis anterior had the most consistent patterns across all walking speeds (Shiavi *et al.*, 1987). The EMG pattern characteristics obtained by the two methods of normalization were similar although the magnitudes of the EMG signals varied.

Despite the similarities in the EMG pattern characteristics between the two methods of normalization, in general, the greatest values in the magnitudes of EMG activity were observed for the data normalized by the mean dynamic method and the inter-subject variability was greater at maximal walking speeds. It is important to point out that the analyzed gait trials demonstrated low intra-subject variability, since all of the coefficient of variation values were lower than 20% (Burden *et al.*, 2003; Portney and Watkins, 2000) at both speeds and normalization methods.

Methods of Normalization

An important issue related to EMG analyses is associated with the method of normalization of the EMG signals (Turker, 1993; Yang and Winter, 1984). The most common employed method is the maximum voluntary isometric contraction (MVIC) (Turker, 1993), however, important limitations are associated with this method. The first one is that the MVIC relies upon a voluntary component, which is even further complicated when the data are collected from subjects with pain and/or muscular coordination problems (Cram *et al.*, 1998), as observed in stroke subjects (Carr and Shepherd, 1998; McComas *et al.*, 1973). Another limitation refers to the values used to normalize the EMG data, which are obtained from a specific situation different from the activity of interest (i.e., gait), besides being time consuming (Cram *et al.*, 1998). Finally, the MVIC method showed the highest values of inter-subject variability in previous studies which compared different methods of normalization of the EMG signals of the lower limb muscles during gait (Burden *et al.*, 2003; Yang and Winter, 1984). As pointed out by Yang and Winter (1984), the normalization of EMG activity of the lower limb muscles during gait, with either the peak or the mean dynamic methods, drastically reduced inter-subject variability and increased the sensitivity of surface EMGs as diagnostic tools for gait analyses.

Considering that the main problem in reporting on EMG activity of lower limb muscles of stroke subjects is the inter-individual variability (Den Otter *et al.*, 2007; Olney and Richards, 1996), only the two methods that have shown the lowest variation values (Burden *et al.*, 2003; Yang and Winter, 1984) were compared.

These methods have also the advantage that the normalized values are derived from walking trials (Yang and Winter, 1984). Based on the observed standard deviation values, the peak method showed the lowest variability for all muscles across all intervals of the analyzed gait cycle. However, analyses of the coefficients of variation, which account for relative differences in the magnitude of the means, revealed that the variability of the data was not homogeneous according to the normalization method.

Except for the rectus femoris which showed similar values, the inter-subject variability of the EMGs on the paretic lower limb at different walking speeds and/or at different intervals of the gait cycle, varied in a non-systematic way with one method which provided lower values

of inter-subject variability than the other. These observations leave future studies uncertain regarding which method to employ and call for other studies.

Similar results were reported by Shiavi *et al.* (1987), who investigated the inter-subject variability of the EMG data of the lower limb muscles of healthy subjects at comfortable walking speeds normalized by the mean and peak dynamic methods. For the rectus femoris, the peak method showed the lowest coefficient of variation values at both gait speeds.

On the other hand, for the gastrocnemius muscles, the mean method showed the lowest variability values at both gait speeds. As previously stated, amongst the four methods of normalization which have been most investigated, the mean and the peak dynamic methods have been shown to provide lower values of inter-individual variability (Burden *et al.*, 2003; Yang and Winter, 1984). Based upon the present results and on those reported by Shiavi *et al.* (1987), when comparisons between the normalization methods were carried out, the peak appeared to be the most appropriate to reduce inter-subject variability of the EMG activity of the rectus femoris, whereas the mean demonstrated to be most appropriate to reduce inter-subject variability of the gastrocnemius muscles during gait, for healthy and stroke subjects, at both comfortable and maximal walking speeds. For the other muscles, it is recommended to perform initial analyses regarding the best method to reduce inter-subject variability.

In agreement with previous studies which compared EMG activities during gait performance of healthy subjects normalized by these two methods, the present results clearly showed that the mean method produced the highest values of EMG activity at both walking speeds. This may be explained by the fact that for this method, lower values were used to normalize the EMG signals compared to the peak method, resulting in the greatest ratio values (Cram *et al.*, 1998).

Interaction Effects Between Walking Speeds and Methods of Normalization

Interactions were found between walking speeds and the methods of normalization for all the assessed gait periods. The differences in EMG activity between comfortable and maximal walking speeds were dependent on the method of normalization.

Therefore, if the aim of a given study is to compare the magnitudes of EMG activity between comfortable and maximal walking speeds, different results would be found depending on the normalization method.

Burden *et al.* (2003) compared four different methods of normalizing EMG signals recorded during the gait of healthy subjects on a treadmill at their normal walking speeds. They also reported similar patterns of muscular activity between the mean and the peak methods.

However, comparisons of the magnitudes of EMG activity were performed only for the isometric and isokinetic maximal voluntary contraction methods of normalization, only in a descriptive manner. The large mean percentage differences between these normalization methods observed by Burden *et al.* (2003) ranged from 21 to 33%.

Since no previous studies were found regarding comparisons of the magnitudes of EMG activity normalized by the employed present methods, it was not possible to conclude that these interactions were only observed for the muscles of the paretic lower limb of stroke subjects.

Therefore, future studies aimed at comparing EMG data during different conditions (walking speeds, pre-post interventions, gait with and without assistive devices) and/or groups, should perform initial analyses of the EMG data considering the various methods of normalization before reporting their main results. This statement must be taken into consideration when the analyses are related to the EMG activity of the paretic lower limb muscles of stroke subjects during gait at maximal speeds. As pointed out, the method of normalization can potentially alter the final results.

EMG Activity at Comfortable and Maximal Walking Speeds

Subjects with a history of stroke are able to increase their walking speeds above their comfortable levels (Bohannon, 1992). The present findings revealed that these subjects were also able to increase the EMG activity of the muscles of the paretic lower limb at maximal walking speeds above the levels observed during comfortable speeds. For all evaluated muscles, the EMG activity was always greater at maximal walking speeds for both methods of normalization.

These findings were similar to those reported by Hesse *et al.* (2001), who investigated the relationships between treadmill speeds and lower limb muscular activity in ambulatory hemiparetic subjects.

They reported positive and significant correlations between these variables (Hesse *et al.*, 2001). Greater walking speeds also increased activation of the paretic lower limb muscles. Similar results were also reported in studies with healthy subjects ((Den Otter *et al.*, 2004; Murray *et al.*, 1984), but the differences in the magnitudes of the EMG activity levels between speeds were considerably lower than those presently found.

Faster walking speeds require greater acceleration and deceleration muscular forces and, thus, are reflected by greater EMG levels. Milot *et al.* (2007) evaluated the paretic lower limb muscles of chronic hemiparetic subjects at comfortable and maximal walking speeds.

They investigated the muscular utilization ratio, an outcome which integrates both muscular strength and gait parameters within the concept of levels of effort, and found that the peak muscular utilization ratios increased with walking speeds.

Besides the greater values of magnitude of the EMG activity at maximal walking speeds, the periods were more clearly determined and the timing periods occurred earlier in the relative stride durations at maximal walking speeds. These results were also supported by Shiavi *et al.* (1987), who described the EMG activity patterns of the lower limb muscles at different walking speeds for healthy subjects.

CONCLUSIONS

Increases in EMG activity with faster walking speeds were associated with more variable patterns of muscular activation. For most muscles and intervals of the analyzed gait cycles, the maximal walking speeds showed the greatest variability values for both methods of normalization.

However, previous studies which compared the EMG activity of the lower limb muscles of healthy subjects at different walking speeds reported that, in general, the variability of the EMG signals decreased as speed increased. In stroke subjects, greater variability in the EMG signals of the paretic lower limb muscles may be associated with the utilization of different muscular strategies to increase speed.

The methods of normalization and the walking speeds modified the magnitudes of EMG activity of the paretic lower limb muscles of stroke subjects and the effects of the methods of normalization were greater at maximal walking speeds. The intra-subject variability was lower for both speeds and normalization methods.

However, the inter-subject variability showed higher values, which were not consistently modified by the method of normalization for the analyzed muscles, except for the rectus femoris. Therefore, it is recommended to perform initial analyses of the EMG data, before choosing the best normalization method to reduce inter-subject variability. These results should be considered when the impacts of locomotor interventions on EMG values with stroke subjects are evaluated.

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Chapter 47

CARDIAC SWINGING CALCIFIED AMORPHOUS TUMORS (SCAT) AND STROKE IN END-STAGE RENAL FAILURE

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ABSTRACT

Cardiac calcified amorphous tumors (cardiac CATs) are unusual non-neoplastic cardiac masses that were originally described in 1997 by Reynolds et al. We recently encountered two patients with a left ventricular cardiac CAT who were successfully treated surgically. Both patients were on hemodialysis for end-stage renal failure and had mitral annular calcification (MAC). In one patient there was massive cardiac calcification. These tumors were fragile, and easily detached from the endocardium. The histological findings were thrombus with angiogenesis and fibrin and calcium deposition. These tumors might be included a special entity of cardiac CATs and may account for some of the strokes or sudden deaths that occur in end-stage renal failure patients who are on hemodialysis. Through the case presentation and literature review, the characteristics and the etiology of the tumor are discussed. The descriptive term of cardiac *swinging calcified amorphous tumor* (cardiac SCAT) is proposed to describe this special risky subgroup of cardiac CATs.

In the Framingham study, mitral annular calcification (MAC) is predictive of a doubling in the risk of stroke after adjustment for multiple risk factors. However, it is still unknown whether the MAC itself is a factor that directly causes stroke or only a marker of increased risk in association with some other unknown factor.

In this chapter, through two clinical cases and literature review, the essence of the cardiac SCAT is discussed.

CASES

Case 1. A 64 y.o. female was referred to our hospital with a diagnosis of pneumonia. She had been on hemodialysis for five years because of diabetic nephropathy. A chest X-ray showed diffuse consolidation in both lung fields. Computed tomography revealed heavily calcified mitral annulus. The leukocyte count was 12,400/ml, and C-reactive protein was 6.4 mg/ml. A blood culture was negative. An echocardiogram showed moderate aortic and mitral regurgitation, and a pedunculated, crotchet shaped mobile tumor that originated from the annulus of the anterior commissure of the mitral valve. The tumor was visualized as a hyper- and homogeneous echo measuring 3 mm x 27 mm (Figure 1). In the systolic phase, it swung widely and its head plunged through the aortic valve into the ascending aorta. A small low-echoic lesion was seen at the center of the tumor head.

Routine echocardiography two months before did not show any evidence of a tumor in the heart. The preoperative diagnosis was infectious endocarditis and pneumonia, and at that time, the tumor was thought to be a vegetation. Because the tumor grew fast, to prevent stroke or systemic embolism, emergency aortic and mitral valve replacement with mechanical valves and tumor resection was performed. Although both valves showed degenerative change, there was no evidence of infectious endocarditis. A soft, fragile, club-shaped tumor that arose from the anterior mitral commissure was easily removed from its origin with forceps without exerting force. There were no other tumors. The postoperative course was uneventful. The patient recovered from pneumonia. She is alive and well with no evidence of recurrence of the tumor as of three years of follow up.

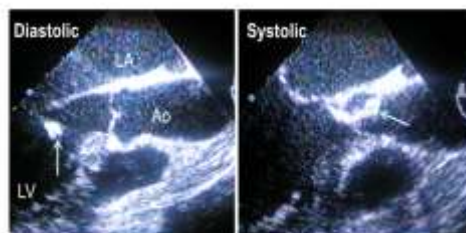
Case 2. A 44 y. o. male who had been on hemodialysis for nine years because of lupus nephritis consulted a cardiologist because routine echocardiography had revealed a mobile tumor in the left ventricle.

Computed tomography showed a densely calcified left ventricle, mitral annulus, and left atrial wall (Figure 2).

Laboratory data showed no evidence of an inflammatory reaction. The tumor was hyperechoic, measured 5 mm x 18 mm, and originated from the base of the anterior papillary muscle (Figure 3). A small low-echoic lesion was seen at the center of the tumor head.

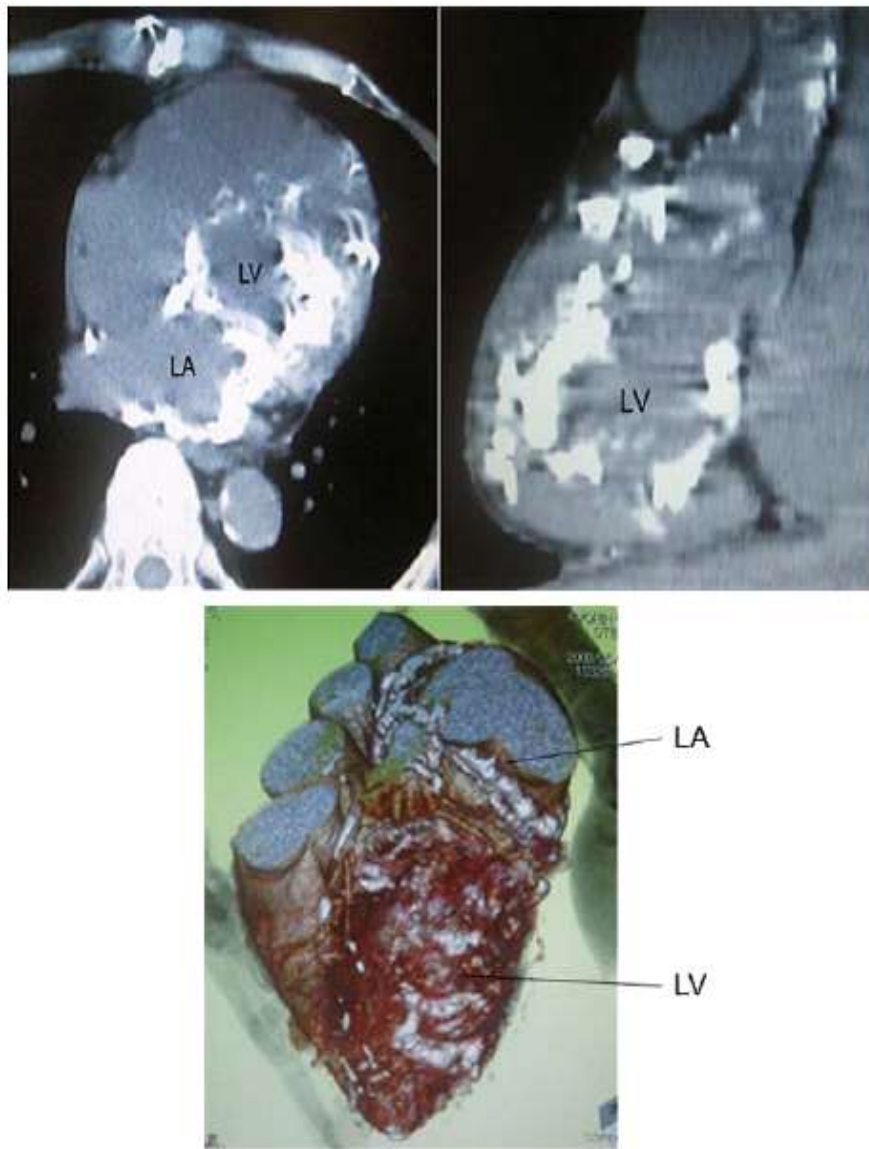
Because follow-up echocardiography 6 weeks later showed that the tumor had grown to 5 mm x 28 mm, elective surgical treatment was performed.

The inside of the left ventricle was visualized with the cardioscope inserted through the orifice of the mitral valve. Three tumors were detected inside the left ventricle (Figure 4).



The head of the tumor was detected in the left ventricle in the diastolic phase (arrow). The tumor plunged through the aortic valve into the ascending aorta in the systolic phase (arrow).

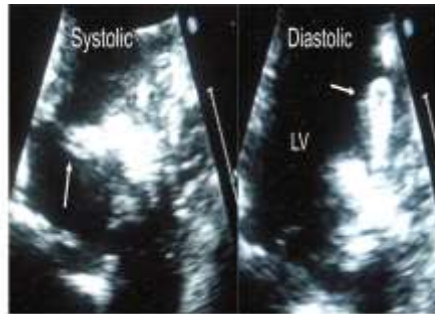
Figure 1. Transesophageal echocardiography.



A computed tomographic scan showed a densely calcified left ventricle, mitral annulus, and left atrial wall. There was no coronary artery stenosis.

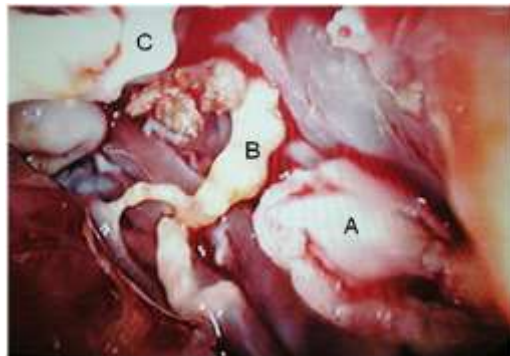
Figure 2. Computed tomography and 3-D computed tomography.

One was a mobile tumor that had been detected preoperatively (A), and the other two were immobile and firmly attached to the left ventricle (B and C). All tumors were resected from their origin. The mobile tumor was fragile and removed from the left ventricular wall without force, whereas the other two tumors were firmly anchored to the tuberculum of the left ventricle, and were resected with scissors. The postoperative course was uneventful. Aortic and mitral valve replacement using mechanical valves was performed because of both stenosis and regurgitation one year after tumor resection. The patient is alive and well with no evidence of the tumor three years after the initial operation.



Echocardiography revealed a mobile swinging pedunculated tumor (arrows) originating from the left ventricle. A small, low echoic area at the center of the tumor head could be detected.

Figure 3.

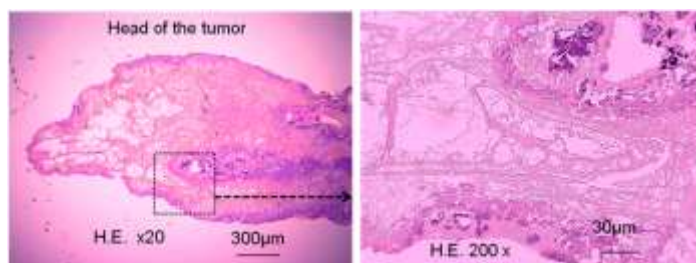


Tumor “A” was a mobile tumor; only the head portion is shown. Tumor “B” and “C” were immobile and firmly attached to the myocardium.

Figure 4. Thoracoscopic view of the left ventricle.

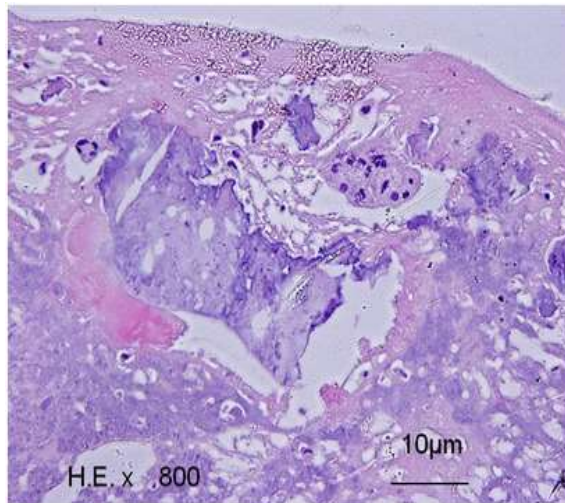
In case 1, pathological examination revealed that the tumor consisted of fibrin. Calcium deposits were detected around the fibrin, but there was no thrombus or capillary angiogenesis (Figure 5). At the proximal part of the stalk, beside the calcified portion, foreign body giant cells were identified (Figure 6).

In case 2, there was red thrombus and capillary angiogenesis in the center of the head of the mobile tumor, which was compatible with the low-echoic lesion. The red thrombus was covered by fibrin and calcium deposition (Figure 7).



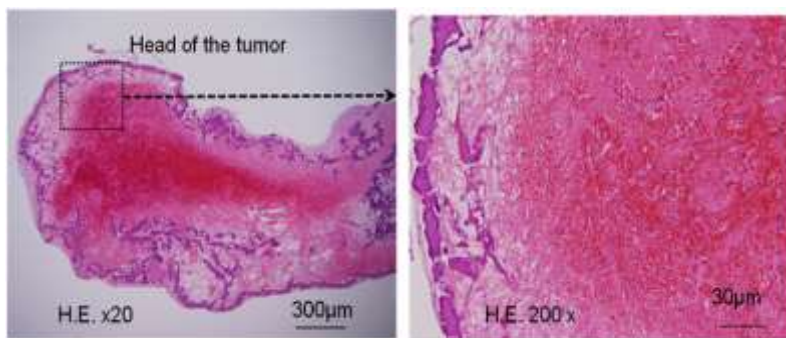
Calcium deposits around the fibrin are seen.

Figure 5. Microscopic appearance of the mobile tumor head in patient 1.



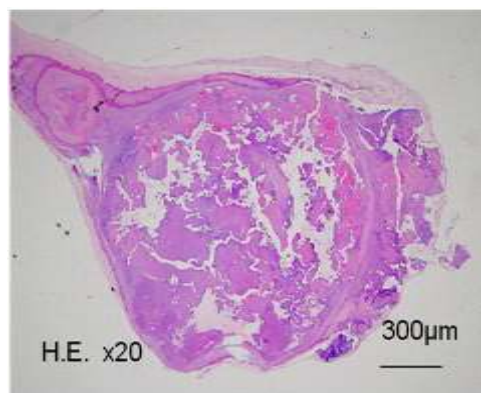
At the proximal part of the stalk, beside the calcified portion, foreign body giant cells were identified.

Figure 6. Microscopic appearance of the mobile tumor stalk in patient 1.



Fibroblasts and capillary angiogenesis are seen in the red thrombus. Fibrin and calcium deposits are seen covering the thrombus.

Figure 7. Microscopic appearance of the mobile tumor head in patient 2.



No thrombus or fibrin was seen. It was characterized by monotonous calcification (= ossification).

Figure 8. Microscopic appearance of the immobile tumor in patient 2.

The two immobile tumors were characterized by monotonous calcification (=ossification) and no any structure, e. g. no thrombus or fibrin (Figure 8).

REVIEW

The cardiac calcified amorphous tumor (cardiac CAT) originally described in 1997 by Reynolds et al. is very rare non-neoplastic cardiac mass, and their etiology, clinical manifestations, and treatment are still unclear [1]. We encountered two cases of cardiac mobile CAT described above. Both two cases were in end-stage renal failure, and required hemodialysis. They also showed severe mitral annular calcification [2]. In the Framingham study, mitral annular calcification (MAC) is predictive of a doubling in the risk of stroke after adjustment for multiple risk factors [3]. However, it is still unknown whether the MAC itself is a factor that directly causes stroke or only a marker of increased risk in association with some other unknown factor. Since the report of a stroke in a patient with MAC by Rytand and Lipstich in 1946 [4], the association between MAC and stroke has been studied by numerous investigators [5-7]. In the Boston Area Anticoagulation Trial for Atrial Fibrillation, MAC was associated with an increased risk of stroke [8]. Postmortem studies have long suggested that MAC may directly cause cerebrovascular accident (CVA) by serving as a source of calcific or thrombotic emboli [9, 10]. Some neuroimaging studies have documented calcific deposits in the cerebral arteries of patients with MAC and CVA [11-13]. In an elderly, longitudinally followed cohort of the Framingham study, Benjamin and coworkers examined the relation between mitral annular calcification and the incidence of stroke in a population-based study [3]. The incidence of stroke during eight years of follow-up was analyzed with a proportional-hazards model adjusting for the mitral annular calcification, age, sex, systolic blood pressure, diabetes mellitus, cigarette smoking, atrial fibrillation, and coronary heart disease or congestive heart failure. As a result, among 1159 subjects whose echocardiograms could be assessed for mitral annular calcification and who had no history or current evidence of stroke at the index examination (51% of all subjects), the prevalence of MAC was 10.3% in the men and 15.8% in the women. Multivariate analysis demonstrated that the presence of MAC was associated with a relative risk of stroke of 2.10 (95% confidence interval, 1.24 to 3.57; $p=0.006$). There was a continuous relation between the incidence of stroke and the severity of MAC; each millimeter of thickening as shown on the echocardiogram represented a relative risk of stroke of 1.24 (95% confidence interval, 1.12 to 1.37; $P<0.001$). Furthermore, even when subjects with coronary heart disease or congestive heart failure were excluded from the analysis, subjects with MAC still had twice the risk of stroke. With these results, they concluded that in an elderly, longitudinally followed population-based cohort, MAC was associated with a doubled risk of stroke, independently of traditional risk factors for stroke. De Bono reported echocardiographic features of extensive MAC in 8 of 151 consecutive patients (5%) with cerebral or retinal ischemic episodes but in none of 188 control patients [14]. Nair et al. followed a cohort of 107 patients with MAC and 107 age- and sex-matched control subjects without MAC were studied and followed for a mean of 4.4 ± 2.4 years. Patients with MAC had higher incidence of cerebrovascular events, occurred in 10% of them as opposed to in 2% in matched control patients [15]. However, it is still unknown whether such calcification contributes directly causally to the risk of stroke or is

merely a marker of increased risk because of its association with other precursors of stroke remains unknown.

Reynolds et al. originally reported 11 cases of cardiac CAT in 1997 [1]. Surgical excision was the treatment of choice in 10 patients. The lesions were firm, yellow-white, and partially calcified, and arose in any of the four chambers. Microscopically, all lesions were similar and composed of nodular deposits or flecks of calcium within a background of eosinophilic, amorphous, sometimes fibrillary material. They hypothesized that it was possible that these masses had been ordinary thrombi that had undergone mummification and calcification rather than organization, at the same time, they posed questions for this hypothesis because none of the tumors showed the characteristic laminations observed in typical organizing thrombi and only three patients had hemosiderin deposition. In this patients series, there was only one patient who had end-stage renal failure as an underlying disease. Although there are no description about tumor mobility or growth speed, only one tumor showed club-shaped (0.3x0.3x1.5 cm) in contrast to the others showed ball shaped. It is noteworthy that this club-shaped tumor was found in a patient who had end-stage renal disease with tumoral calcified left ventricle (the site was undescribed). Pathologically, giant cell was detected only in this patient.

Tsuchihashi et al. reported four cases of rapidly growing mobile cardiac calcinosis accompanied by MAC [16]. One of the patients had right hemiplegia, and another died suddenly after four months of follow up. One patient died of acute myocardial infarction secondary to multivessel disease, and surgical resection was performed in the fourth patient.

A few older postmortem case reports and studies suggested a pathophysiologic link between MAC and stroke. Ulceration of the mitral annulus and extrusion of calcium through the overlapping cusp was observed by Pomerance in 5% of 258 autopsy cases [9], and calcific emboli to the brain and other organs from ulcerated MAC were then reported by Ridolfi and Hutchins [17].

In 1995, Stein and Soble described two patients with cerebral embolism who had mitral valve calcification and a soft mobile mass that appeared to be thrombus on the calcified portion of the mitral apparatus [18], and they confirmed resolution of the masses in both patients after antiplatelet and anticoagulation therapy using Aspirin and Warfarin. They concluded that these cases supported the hypothesis that thrombus formation is a pathophysiological link between ischemic cerebral events and MAC in some patients. They mentioned that whether antiplatelet or anticoagulant therapy might prevent cerebral embolism in patients with MAC is unknown and is worthy for further study.

Willens et al. treated rapidly progressing mobile components associated with MAC in patients with chronic renal failure with the phosphorus lowering agent sevelamer HCL and aspirin and obtained resolution. Eicher et al. focused on the echocardiographic findings and the association between MAC and risk of thromboembolism [19]. Among 182 patients who underwent both transthoracic (TTE) and transesophageal echocardiography (TEE) after an arterial thromboembolic event, they identified 10 patients (5.5%) who had MAC and no any other potential embolic substrate. In 3 of them (1.64%), TEE disclosed a long, pedunculated, vegetation-like mass attached to the posterior part of a heavily calcified fibrotic mitral annulus. All 3 patients were treated by anticoagulation with aspirin or dipyridamole concomitant with fluindione. Since regression and complete resolution were achieved, they proposed that when a mitral annular thrombus is diagnosed, anticoagulation therapy and antithrombotic agents should be given for a synergistic effect. They also suggested

pedunculated thrombi of the mitral annulus are a possible “missing link” between MAC and stroke.

Although some reports try to distinguish thrombi from CATs, the pathological findings in our two cases and the courses of the reported clinical cases described above suggest that, cardiac CAT may be the late phase of chronological changes of thrombus in the early phase. The reason why cardiac CATs originate mainly from MAC is unclear. Considering that MAC occurs in >40% of the patients with end-stage renal disease, as our second case shows, they not only originates from MAC but from everywhere the calcified part of the heart and MAC is only the most popular portion to be calcified [20]. It is not clearly understood why the cardiac base is vulnerable to calcium deposits. In extraskeletal calcinosis, Ca-P deposits on the multifollicular fibrous wall are also seen frequently in the hyperkinetic joint capsule. The similarity of the histological and pathophysiological background of the target tissue may explain the deposit of fibrous tissue in cardiac base. But the clinical significance of cardiac calcinosis is very different from joint calcinosis. Injury and erosion of the endocardium or myocardium caused by calcium deposition and inflammation may be related to the initial development of the CAT. Continuously, with the expose to the bloodstream, thrombi with capillary angiogenesis grow with the fibrin exudation and calcium deposition. Secondary hyperparathyroidism may also plays a key role in the cause of cardiac calcification, where the $\text{Ca} \times \text{P}$ product is <50 [21]. Serum magnesium, vitamin D, and serum aluminum are all influential factors. A further symptom of rapid cardiac calcinosis is hypoparathyroidism. Patients on hemodialysis with hyper parathyroidism had a higher incidence of metastatic calcification, which resulted from the decreased reservoir function of bone for absorbed calcium and phosphate from the intestine [22]. Moreover, generally administered calcium and active vitamin D₃ could exacerbate hypercalcemia, and might increase the risk of tumoral calcinosis in the cardiovascular system of patients with end stage renal disease and hypoparathyroidism. As the calcium deposition progresses over time, the capillary vessels, fibrin, and thrombus may disappear. Abnormal calcium and phosphate metabolism due to chronic renal dysfunction and the inflammatory state associated with hemodialysis may contribute to the endothelial erosion postulated to underline these rapid growth and rapid pathological change [23]. The appearance of the foreign body giant cells may represent the relationship with the immunological and inflammatory reaction. However, we cannot find any reason to explain why there are two types of calcified tumor in even though the same patient as case 2: immobile firmly attached one to the heart or mobile, clubbed shaped, rapid growing and fragile tumor.

The definition of cardiac CAT is still in vague. In the literature, cardiac CATs are described as consisting of a wide spectrum of tumor morphologies and pathological characteristics. A case report described a case of diffuse calcific infiltration of the left ventricular myocardium without mobile component as a cardiac CAT [24]. Other authors reported a right ventricular nonmobile calcified 1.5-cm mass involving the annulus of the tricuspid valve as a cardiac CAT [25]. Massive cardiac calcification, cardiac calcified mass or porcelain heart may be suitable expression to describe such tumorous calcification [26]. Based on the intraoperative findings in our two cases and literature review, mobile CATs that exhibited a swinging motion clearly indicate an impending risk of stroke, myocardial infarction, or other systemic embolism. They may occupy some part of the possible “missing link” between MAC and stroke in hemodialyzed end-stage renal disease patients.

Thus, mobile, swinging cardiac CATs should be regarded as distinct from the porcelain heart or immobile cardiac CAT. We propose the descriptive term of *swinging calcified amorphous tumor* (SCAT) to describe this high risk subgroup of cardiac CAT. SCATs are soft, fragile tumor that easily detach from the endocardium, whereas immobile CATs are firmly anchored to the myocardium. They are usually found in patients who require hemodialysis, and originate from any calcified part of the heart. MAC is known as the main site, however, MAC is not the specific site as their origin. As differential diagnosis, other cardiac masses e.g., myxoma, fibroelastoma, vegetation due to infectious endocarditis, malignant cardiac tumors etc. are listed. Especially, calcified tumors e.g., calcified myocardial tuberculomas, tephaceous pseudogout, and osteosarcoma should be carefully differentiated [27].

The incidence of this special category of intracardiac tumor is unclear. However, in view of fact that two cases were treated in same year at a single institution, awareness of this tumor and routine echocardiographic evaluation of patients with MAC or cardiac calcification who are on hemodialysis may lead to the discovery of more cases. Further serological and pathological investigation will reveal the mechanism of the development, growth, and chronological changes in SCATs. Anticoagulation therapy and antithrombotic agents may affect not only on the outside of the tumor but from inside via capillary vessels. As cases accumulate, proper protocol of treatment will be established.

CONCLUSION

In conclusion, routine follow-up echocardiography is recommended for the patients with MAC or cardiac calcification who are on hemodialysis. Although the efficacy of a preventive anticoagulant is a matter of controversy, immediate anticoagulation, anti-inflammation and thrombolytic therapy should be started whenever a mobile hyperechoic tumor originating from a calcified site, especially in the left-side of the heart is detected. The indications for emergency surgery are also a matter of controversy, when a tumor exhibits rapid growth despite appropriate medical therapy, complete surgical resection should be considered to prevent strokes or sudden death in patients with end-stage renal failure.

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Chapter 48

ENDOVASCULAR TREATMENT OF
ACUTE ISCHEMIC STROKE

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INTRODUCTION

Stroke is one of the most frequent causes of mortality, morbidity and disability of population in developed countries. [1, 2] Ischemic stroke (IS) represents 80 - 85% of all strokes in Europe and Northern America. The most often cause of IS is an acute occlusion of cerebral arteries, which can be demonstrated in more than 70% of patients in the first three to six hours after onset of symptoms. [3] Mortality of IS patients during the first month ranges between 10% and 17% and even up to 75% in patients with malignant infarction. [4] Finally, only about 30% of IS patients are independent after three months. [2] These facts support the importance of IS.

Table 1. Thrombectomy devices for acute stroke in use [14].

Device Class	Company Name	FDA Indication	In Clinical Use?
<i>Aspiration/Suction</i>			
Amplatz Thrombectomy	Ev3 Medical	Mechanical dissolution of thrombus within dialysis fistulae	No longer marketed
AngioJet	Possis	Breaking apart or removing of thrombus in peripheral veins or arterio-venous access conduits	Yes
NeuroJet	Possis	N/A	No longer marketed
Oasis Thrombectomy	Boston Scientific	Removing thrombus from hemodialysis access grafts	No longer marketed
Penumbra	Penumbra, Inc	Revascularization of patients with acute ischemic stroke	Yes
Vasco +35	Balt Extrusion	N/A	Not in U.S.
<i>Clot Retriever</i>			
Attractor-18	Boston Scientific	N/A	No longer marketed
Catch	Balt Extrusion	N/A	Not in U.S.

Table 1. (Continued)

Device Class	Company Name	FDA Indication	In Clinical Use?
In-Time	Boston Scientific	Retrieval of intravascular foreign objects in peripheral vascular, neurovasculature and cardiovascular	No longer marketed
MERCI	Concentric Medical	Restore blood flow in the neurovasculature	Yes
Phenox	Phenox GmbH	N/A	Not in U.S.
TriSpan	Boston Scientific	N/A	No longer marketed
<i>Ultrasonography</i>			
EKOS	EKOS Corporation	Infusion of fluids into peripheral vasculature	Yes
OmniWave	OmniSonics	Removal of thrombus and infusion of fluids into peripheral vasculature	No longer marketed
<i>Snare</i>			
Alligator	Chestnut Medici Technologies, Inc.	Peripheral and neurovasculature foreign body removal	Yes
Amplatz Gooseneck	Ev3 Medical	Retrieval and manipulation of atraumatic foreign bodls in coronary and peripheral cardiovascular system and the extra-cranial neurovascular anatomy	Yes
EnSnare Device	Merit Medici Systems, Inc.	Retrieval and manipulation of foreign objects in the cardiovascular system or hollow viscous	Yes
Device Class	Company Name	FDA Indication	In Clinical Use?
Neuronet	Boston Scientific	N/A	No longer marketed
Soutenir	Solution	N/A	Not in U.S.
<i>Laser</i>			
EPAR	Endovasix Inc.	N/A	No longer marketed
LaTIS	Spectranetics	Removal of thrombus from vascular grafts	No longer marketed

EPAR - Endovascular Photoacoustic Recanalization, FDA—Food and Drug Administration, NA—not applicable, U.S.—United States

The location of cerebral artery occlusion and time to recanalization are known independent prognostic factors of IS. Early recanalization within six hours after onset of symptoms is associated with a significantly higher chance of independency after 90 days with a significant reduction of mortality [5].

In the last decade, the number of methods used for acceleration of artery recanalization strongly increased. In addition to pharmacological methods, especially intravenous (IVT) and intra-arterial thrombolysis (IAT) [6-8], mechanical (neuro-interventional) methods (i.e., Merci Retrieval System®, Penumbra System®, Solitaire® stent, Trevo Pro®, Catch device®, Phenox Clot Retriever®, BONNET Intracranial Flow Restoration Device®, pRESET Thrombectomy Retriever®, direct stent placement, EkoSonic™ Endovascular System, In-Time™ Retrieval Device, Amplatz Goose-Neck MicroSnare®, Attractor-18™, Neuronet™ Guidant device, LaTIS laser device®, Possis Angiojet system®) were tested and introduced into clinical practice similarly as in the treatment of heart ischemic syndromes—Table 1 [9-14].

Data from the meta-analysis of 53 clinical trials (including 2,066 patients) suggests that early recanalization is present only in 24.1% patients without specific treatment (spontaneous recanalization), 46.2% patients treated with IVT, 63.2% patients treated with IAT, 67.5% of patients treated with combined IVT - IAT and in up to 83.6% patients treated with mechanical methods [5]. Endovascular therapy as a new early recanalization option for patients with acute IS is currently intensely investigated. Nevertheless, the use of these methods only in specialized centers represents the main limitation.

Several thrombectomy devices will be introduced with results of studies and a list of ongoing trials.

THROMBECTOMY DEVICES

Merci Retriever (Concentric Medical, Mountain View, CA, U.S.)

The Merci Retrieval System® is a minimally invasive catheter-based system designed to retrieve and remove clots in patients experiencing acute IS. It was one of the first thrombectomy systems used in acute stroke patients. The Merci Retrieval System® consists of three parts: Merci retriever, Merci microcatheter and Merci balloon guide catheter—Figure 1.

The Merci Retrievers® are constructed from nitinol (nickel titanium) memory-wire and are corkscrew-shaped. This design is considered to be best suited for blood clot removal from large vessels within the neurovasculature. The Merci Retriever® is delivered through the dedicated Merci® MC18L microcatheter to the lesion location in its linear formation. Once deployed, the retriever returns to its coiled shape to ensnare and retrieve the clot. The Merci Retrievers are compatible with the Merci® Balloon Guide Catheter. This catheter comes in two sizes—namely 8 and 9F. The catheter has compliant silicone balloon mounted at its end. The balloon soft and atraumatic design provides temporary flow arrest in thrombectomy procedures when inflated. This should help to prevent distal embolization. One lumen is used to inflate and deflate the balloon, while the second lumen allows continuous access to the neuro vasculature.

There are currently ten Merci Retrievers® available to match different vessel sizes. The newest generation of Retrievers®, the V Series, incorporates features of the two earlier generations to create a best-in-class thrombectomy device. The V Series is designed such that the proximal loops stretch to engage the clot, while the two distal loops remain intact to capture the clot.

Merci Retrievers® are also indicated for use in the retrieval of foreign bodies misplaced during interventional radiological procedures in the neuro, peripheral and coronary vasculature.

The Merci Retriever® was first cleared for human use in 2004, by the Food and Drug Administration (FDA). Since 2004, the Merci Retriever® has been used in more than 14,000 patients throughout the world including the United States, Europe, Australia, Singapore and Canada, and since May 2010, also in Japan.

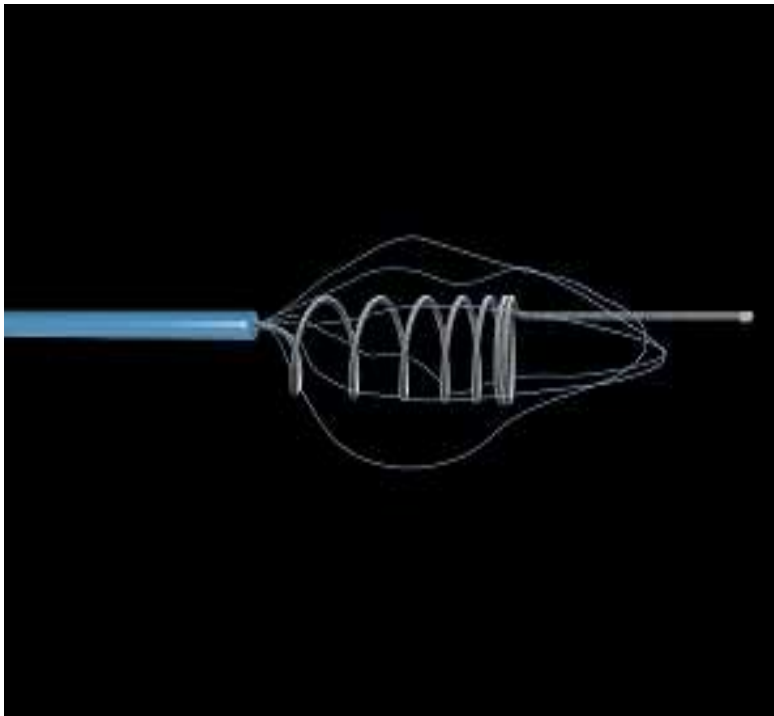


Figure 1. Merci V Retriever® (Concentric Medical, Mountain View, CA, U.S.).

Published Studies

Successive generations of the MERCI device have been reported in three prospective, single-arm, nonrandomized, multicenter feasibility and safety studies. In the MERCI-I trial, 28 patients were treated with this technique with 64% successful recanalizations. In the following prospective, nonrandomized, multicenter MERCI II trial, the safety and efficacy of a novel embolectomy device (Merci Retriever) to open occluded intracranial large vessels within eight hours of the onset of stroke symptoms were tested. All patients were ineligible for intravenous recombinant tissue plasminogen activator (rt-PA). Recanalization was achieved in 46% of patients on intention to treat analysis. This rate was significantly higher than 18% in historical controls of ($P < 0.0001$). Clinically significant procedural complications occurred in 7.1% patients. Symptomatic intracranial hemorrhages (SICH) were observed in 7.8% patients. Good neurological outcomes (defined as a modified Rankin scale [mRS] 0-2) were observed in 46% patients versus 10% in historical controls (relative risk 4.4, $P < 0.0001$) and mortality was in 32% patients versus 54% in historical controls (relative risk 0.59, $P < 0.01$) [15].

The results were confirmed in the Multi MERCI international, multicenter, prospective, single-arm trial. One hundred and sixty-four patients with large vessel IS who had received IV rt-PA but did not recanalize or who were ineligible for IV rt-PA and were treated within eight hours of symptom onset were included in the study. Treatment with the Retriever alone resulted in successful recanalization in 57.3% treatable vessels and in 69.5% after adjunctive

therapy (IA rt-PA, mechanical). SICH occurred in 9.0%. Clinically significant procedural complications occurred in 5.5% patients [16]. There was no definite association between time to treatment or reperfusion and outcomes in the unadjusted analysis. After adjustment for age, baseline NIHSS, and glucose, there was a strong trend toward fewer independent outcomes with later reperfusion times [17].

Final recanalization status was the strongest predictor of clinical outcomes in patients undergoing thrombectomy in presented studies. The ability to remove the clot was negatively influenced by systolic blood pressure [18].

Post hoc analysis with patients in the MERCI (68 patients) and Multi MERCI (81 patients) trials, who would have been eligible for PROACT II, showed a trend toward better clinical outcomes compared to the PROACT II control arm. In both unadjusted and adjusted analyses, mortality rates did not significantly differ between embolectomy patients and PROACT II control patients [19]. No statistically significant differences were found between patients with occlusion of M2-segment of middle cerebral artery (MCA) and M1-segment of MCA for symptomatic hemorrhage, clinically significant procedural adverse events, favorable 90-day outcome, or 90-day mortality. In multivariate analysis, final revascularization was the strongest independent predictor of good outcome at 90 days [20].

Further analysis showed that mechanical thrombectomy of acute intracranial internal carotid artery (ICA) occlusion using the Merci Retriever device, alone or in combination with adjunctive endovascular therapy, has a high rate of successful vessel recanalization (39%). Subjects with successful ICA recanalization by this method have improved post-stroke clinical outcome and survival compared with subjects in whom the ICA is not successfully recanalized [21]. Patients with vertebrobasilar occlusion in the MERCI and Multi-MERCI trials achieved recanalization in 78% cases, mortality was 44% and good outcomes were seen in 41%. Outcomes in these patients compared favorably with natural history reports. Patients with recanalization tended to have better outcomes than those without [22].

Penumbra System® (Penumbra Inc., Alameda, CA, U.S.)

Penumbra Inc.'s IS device platform, the Penumbra System® (Figure 2), is designed to revascularize or open up a blood vessel that is blocked by a blood clot. The Penumbra System® is FDA cleared and CE-marked. The system is based on a continuous aspiration thrombectomy platform. It includes reperfusion microcatheter that is connected to an aspiration pump through aspiration tubing, generating a suction force of -700 mm Hg. A teardrop-shaped separator is advanced and retracted within the lumen of the reperfusion catheter to debulk the clot for ease of aspiration. As a nonpharmacologic tool, it has the potential of reopening a vessel without the use of adjunctive thrombolytics, thus offering dual options for recanalization via a single access platform. In addition, the system is designed to minimize the need to blindly penetrate into the occluded vascular segment because it operates from the proximal end of the clot. Due to the flexibility and variety of available sizes (0.26 – 0.54 inch) of the reperfusion microcatheters, even smaller distal branches such as M2 and A2 can be successfully accessed for recanalization.

Published Studies

The phase 1 trial showed a 100% revascularization rate to TIMI 2 or 3 in 20 patients (21 vessels). The pivotal phase 2 trial, completed in September 2007, showed a TIMI 2 or 3 recanalization rate of 81.6% with a low periprocedural serious adverse event score of 3.4% in 125 patients treated with this device; none were device-related. SICH occurred in 11.2% of the patients, and all-cause mortality was 32.8%. Good neurologic outcome as (defined as a > 4 points improvement on the NIHSS score at discharge) was observed in 57.8% of the patients, whereas good functional outcome as defined by a 90-day mRS score of zero to two was reported in 25% of patients. Patients who were successfully recanalized by the Penumbra System® had generally better outcomes than those who were not [23,24].



Figure 2. Penumbra System® (Penumbra Inc., Alameda, CA, U.S.).

First single-center experiences as well as preliminary data from the first post-market multicenter study seemed to confirm these findings. In a retrospective case review study performed in 139 patients treated by the Penumbra System® at seven centers in the United States and Europe, the device was found to successfully recanalize the target vessel in 84% of cases, with similar outcome and intracranial hemorrhage rates as those in the pivotal trial. In fact, 40% of the patients had achieved a mRS score of ≤ 2 at 90 days, which is a significantly higher rate than that reported earlier [25]. Thus, it appears that the favorable safety and effectiveness profile of the Penumbra System® observed in the pivotal trial can be extended to the real-world setting.

Solitaire™ Stent (Ev3, Irvine, CA, U.S.)

Solitaire™ AB (FR) Neurovascular Remodeling Device is the only fully deployable and completely retrievable self-expanding stent designed for bridging the neck of aneurysms. It can be fully retrieved, even when fully deployed, repositioned and redeployed again—Figures 3 a,b.

Solitaire™ AB (FR) stent is deployed through a standard 0.021" or 0.027" micro catheter on a 0.016" pushwire, which means that Solitaire™ AB (FR) delivers just like an intracranial coil. It allows multiple retrievals, even after full deployment for adjustment and superior placement. It can be introduced easily even into very tortuous vessels. Its open slit, closed-cell design gives Solitaire AB an optimal radial force with good kink resistance. It can be deployed by electrolytic detachment. It is available in two diameters of 4 or 6 mm and lengths 26, 31 and 41 mm.

As per the manufacturer's recommendation, this stent is designed for the treatment of intracranial neurovascular disease. At first, it was intended for wide neck aneurysm treatment

only. At present, it is also renowned for its excellent results in intracranial vessel recanalization in stroke patients.

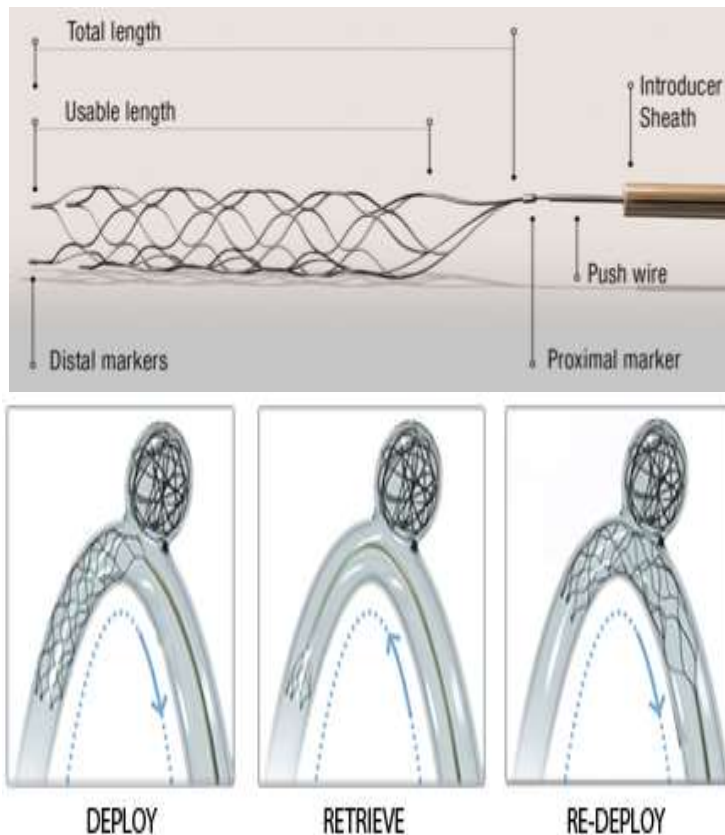


Figure 3 a,b. Solitaire™ AB (FR) Neurovascular Remodeling Device (Ev3, Irvine, CA, U.S.).

The unique Solitaire™ stent design features allow dual functionality: firstly, it acts as a temporary intracranial bypass providing immediate flow restoration through the thrombus after the temporary stent deployment. Secondly, it acts as a clot retriever, trapping thrombus into its cells allowing the clot removal. It received CE Mark approval as a flow restoration device.

Procedure

All procedures are performed via a femoral artery approach under sedation or in general anesthesia in extremely agitated patients. For the anterior circulation, an 8 Fr or 9 Fr Merci balloon guiding catheter (Concentric Medical, Mountain View, CA, U.S.) is introduced through a femoral sheath into the appropriate carotid artery. For the posterior circulation, a 6 Fr Envoy guiding catheter (Cordis, Miami Lake, FL, USA) is placed into the dominant, or navigable, vertebral artery. A 0.021 inch internal diameter microcatheter Prowler Select Plus (Cordis) or Vasco 21 (Balt, Montmorency, France) is navigated distally to the point of

occlusion over a 0.014 inch steerable microwire. A microcatheter angiographic run is then carried out in order to confirm and define the vasculature distal to the thrombus.

The Solitaire™ AB (FR) is introduced through the 0,021" microcatheter, and the device is deployed across the occluding thrombus. The microcatheter is then partially withdrawn, so that the distal marker is positioned at the exact level of the proximal marker of the stent. After the Solitaire™ AB (FR) deployment, an angiographic control is performed in order to evaluate the correct placement and expansion of the stent and to find out if there already is flow restoration past the occlusion site. After the angiographic run, the Solitaire™ AB (FR) is maintained in place for a few (three to seven) minutes to allow device expansion. After this time, the stent is slightly resheathed and then both the deployed Solitaire™ AB and the delivery microcatheter are gently pulled back together and recovered through the guiding catheter. Immediately after the retrieval, it is necessary to aspirate the content of the guiding catheter in order to remove any clot debris eventually present in the lumen of the guiding catheter. In case of unsuccessful thrombus removal, another attempt is usually indicated. After thorough cleaning of the stent, it can be reintroduced into occlusion site. If necessary, up to five passes are performed using the device. The integrity of the stent must be consistently checked after every pass. In case of repeated unsuccessful thrombus retrieval, suboptimal angiographic result in atherosclerotic lesions or inability to recapture the stent, a bail-out option of repositioning the stent is available following with permanent deploying it by electrolytic detachment, similar to coil. However, its radial force is not very high, and very often different types of stents need to be used to finish the procedure.

Mechanical thrombectomy also has its specific potential drawbacks. It usually takes a longer time compared to direct stenting. There is also increased risk of intimal injury to the vessel wall while withdrawing an unfolded stent to perform thrombectomy. It luckily proved to be of little concern in the clinical setting. However, microscopic studies have proved some minor intimal injuries in animal studies. Vasospasms and subarachnoidal hemorrhage have been described in clinical studies after thrombectomy with the Solitaire device.

Published Studies

Current reports with Solitaire device come from different European centers since the device is not currently approved in the United States. Castano et al. [26] reported their experience in the treatment of 20 patients with acute IS and large vessel occlusion in the anterior circulation. Recanalization grade TICI 2b/3 was achieved in 90% of treated vessels, and no procedural complications were reported. SICH occurred in 10% of patients, and 45% of them achieved a mRS ≤ 2 at three months. The device used in this study was the Solitaire AB stent, initially designed as an aneurysm neck bridging stent. The device was deployed for one or two min before retrieving it to perform embolectomy. Recanalization required in average 1.4 passes. The successful off-label use of this stent prompted the creation of the Solitaire™ FR device with minimal modifications from the AB version and specifically designed for treatment of acute IS.

Machi et al. [27] achieved successful recanalization in 50 out of the 56 patients (89%) treated with the Solitaire™ FR device. Procedure-related complications occurred in 9% of patients, and 46% of them achieved a mRS ≤ 2 at discharge. The mean number of passes required per procedure was two (1–5 range). The device was positioned across the area of

occlusion for three to seven min and then gently recovered into the guide catheter. Vasospasm during retrieval of the fully deployed stent was a common phenomenon; two patients had asymptomatic subarachnoidal hemorrhage and one patient, SICH.

Similarly, Roth et al. [28] treated 22 patients with acute IS and achieved TICI flow 2a/b or 3 in 91% of patients. Two SICH occurred, and 50% of patients achieved a mRS ≤ 2 at 90 days. Vasospasm after thrombectomy was noticed in three patients, and no device-related complications were reported.

Seifert et al. [29] reported experience with a Solitaire™ device in conjunction with IVT or IAT in four patients, achieving TIMI grade 2 to 3 recanalization in all patients and clinical improvement (median mRS, one at 30 days) in 50%. There was one death, and the other patient was lost to follow-up.

Investigators of the REscue, COmbined, and Stand-alone Thrombectomy (RECOST) study achieved TICI 3 flow in 84% of patients with acute IS treated with the Solitaire™ FR/AB device. [30] The aim of this study was to evaluate and appraise the timing, safety and efficacy of an integrated stroke management protocol. At three months, 54% of patients achieved a mRS ≤ 2 and 2% had SICH. The device was deployed for two to seven min in the target occlusion before thrombectomy.

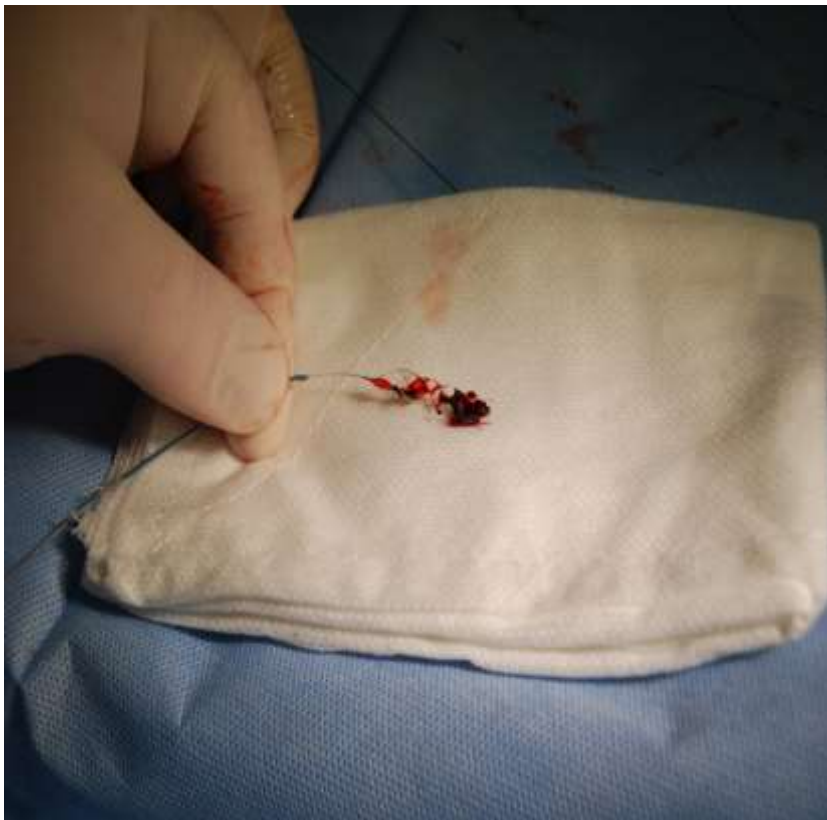
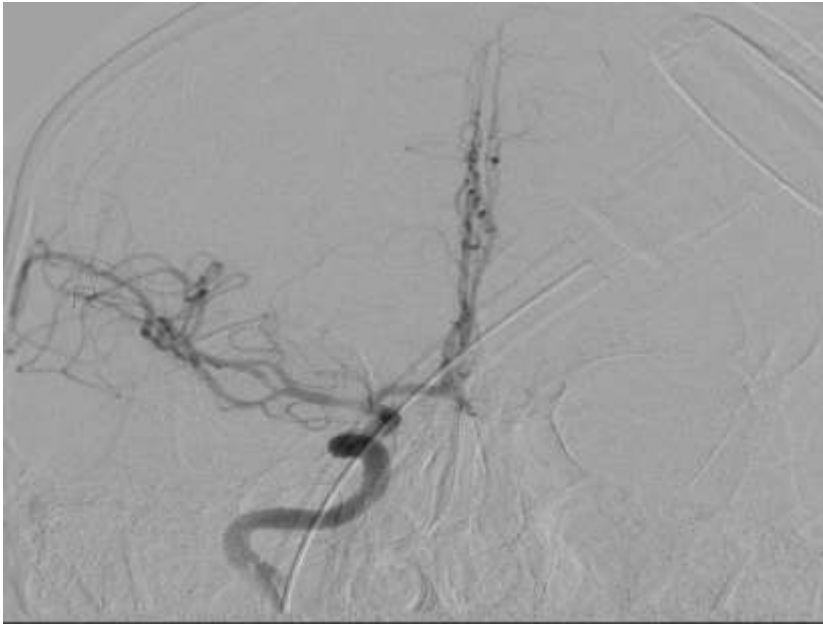
CASE REPORT 1

A 74-year-old male was admitted to the hospital (stroke center) with left-sided hemiparesis, left-side facial palsy, dysarthria, eye deviation and neglect syndrome (NIHSS 14 points). Computed tomography angiography (CTA) finding confirmed T-occlusion of the distal ICA, anterior cerebral artery (ACA) and middle cerebral artery (MCA) on the right side. Atrial fibrillation treated with Aspirin® 100 mg daily, arterial hypertension and hypercholesterolemia were in medical history. Patient was treated with IVT (90 mg of rt-PA) 140 minutes after stroke onset. Patient's clinical status did not improve during IVT; therefore, he was transferred to angiography for mechanical recanalization. Persistent T-occlusion of the ICA was detected (Figure 4a). Successful thrombectomy with Solitaire™ device was performed, and the vessels were recanalized completely five hours after onset of symptoms (Figures 4 b-d). At discharge, patient presented with residual mild left-sided hemiparesis (mRS 2); small ischemic area in the right basal ganglia was shown by computer tomography (CT).

Trevo Pro® (Concentric Medical, Mountain View, CA, U.S.)

The Trevo System® is a non-deployable, sized 4 x 20mm stent-like device currently used in Europe and Canada. Its body is very soft and allows easy navigation through tortuous vessels, and its distal closed-end is intended to prevent vessel perforation—Figures 5 a,b. The device can be used in vessels ranging from 1.5 to 3.5 mm in diameter. The orientation of the stent struts has the thinner portion of the struts facing the vessel lumen in order to optimize thrombus incorporation, opposite to the design of stents used to treat intracranial aneurysms or intracranial atherosclerosis.





Figures 4 a-d. Recanalization of middle cerebral artery using Solitaire™ stent: Distal occlusion of internal carotid artery (4a). Solitaire stent deployed in the middle cerebral artery - flow restoration also called temporary bypass (4b). Middle cerebral artery recanalization after successful thrombectomy (4c). Solitaire stent immediately after extraction with thrombi in stent struts (4d).

The Trevo System® is being introduced into the occluded vessel via a 0.021” inner diameter large microcatheter. The deployment and clot extraction should be performed in a similar manner to that of Solitaire™ stent. The system is currently under investigation in pivotal study named Trevo 2 Clinical Trial. [31]

Mendonça et al. published results of a prospective study with 13 acute stroke patients (eight patients with MCA and five patients with ICA occlusion) treated by Trevo system®. Revascularization was achieved in 77% patients without periprocedural complications. Four patients (30%) died during the 90-day follow-up period and four patients (30%) achieved functional independence [32].

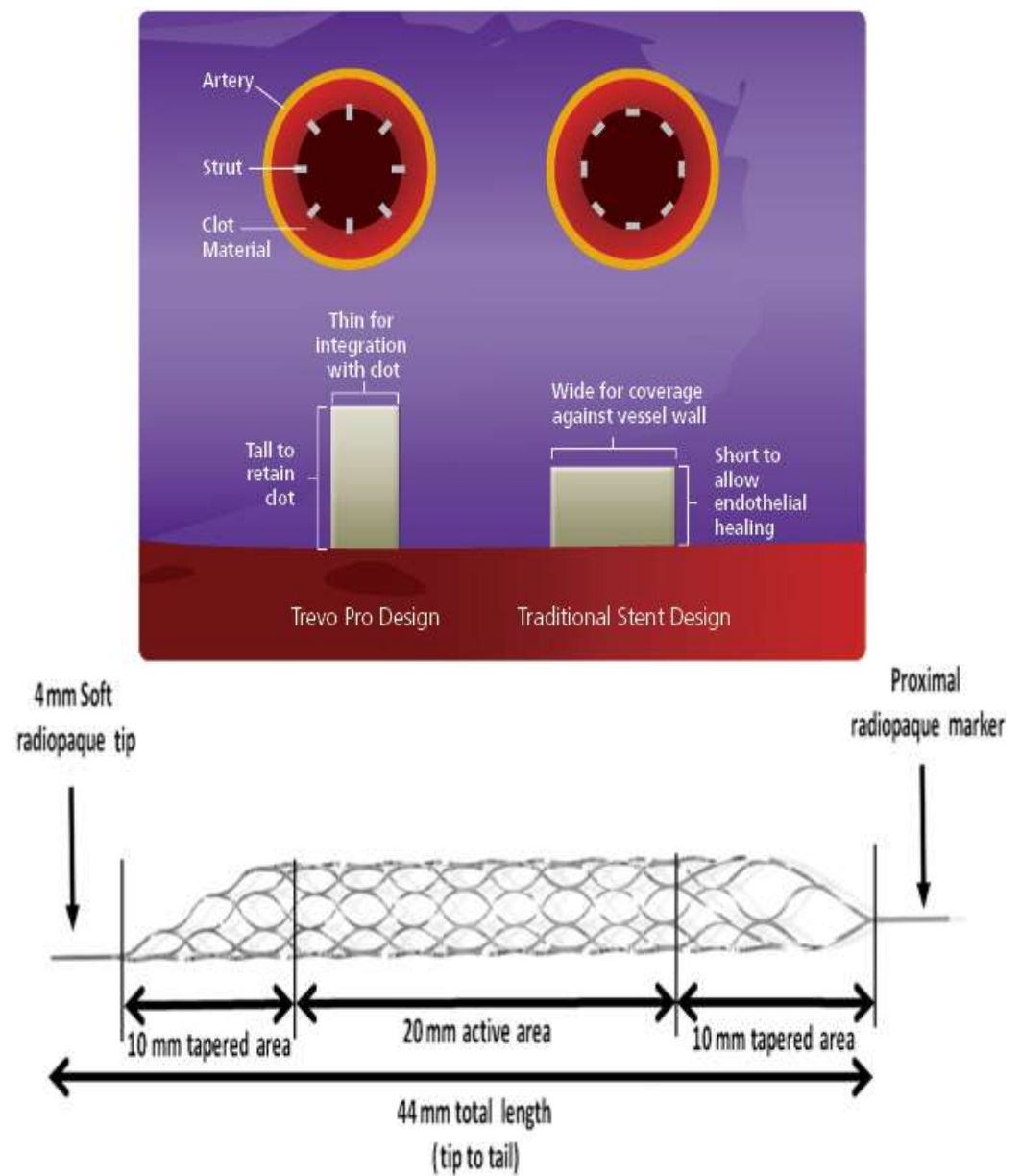


Figure 5 a,b. Trevo Pro® (Concentric Medical, Mountain View, CA, U.S.).

2.5 Catch Device® (Balt Extrusion, Montmorency, France)

The Catch Device® owes its design to Leo stent and is made of Nitinol. In fact, it looks like tiny wire basket intended for thrombus retrieval—Figure 6. Its external diameter is 4 millimeters, and the device is intended for use in vessels of diameter up to 5 millimeters. This device must be introduced exclusively by microcatheter Vasco+21, which has internal diameter of 0.0236". The microcatheter must be positioned at least 2 cm downstream behind the thrombus. Then the basket is opened, and the whole system can be withdrawn through 6F guiding catheter. However, the Catch basket cannot be resheathed into the Vasco microcatheter. Mourand et al. published results of retrospective study with 40 consecutive acute IS patients. Recanalization (TIMI grade 2 – 3) was achieved in 65% cases. SICH occurred in 18% cases. Periprocedural complications occurred in 15% patients without clinical consequences: four clot fragmentations and two vasospasms. Good functional outcome (mRS ≤ 2) after 90 days was achieved in 39% patients. Good functional outcome at 90 days was more frequent (56.5 vs. 7.7%), and mortality rate was lower (30.0 vs. 61.5%) in patients with successful recanalization in comparison with patients with unsuccessful recanalization [33].

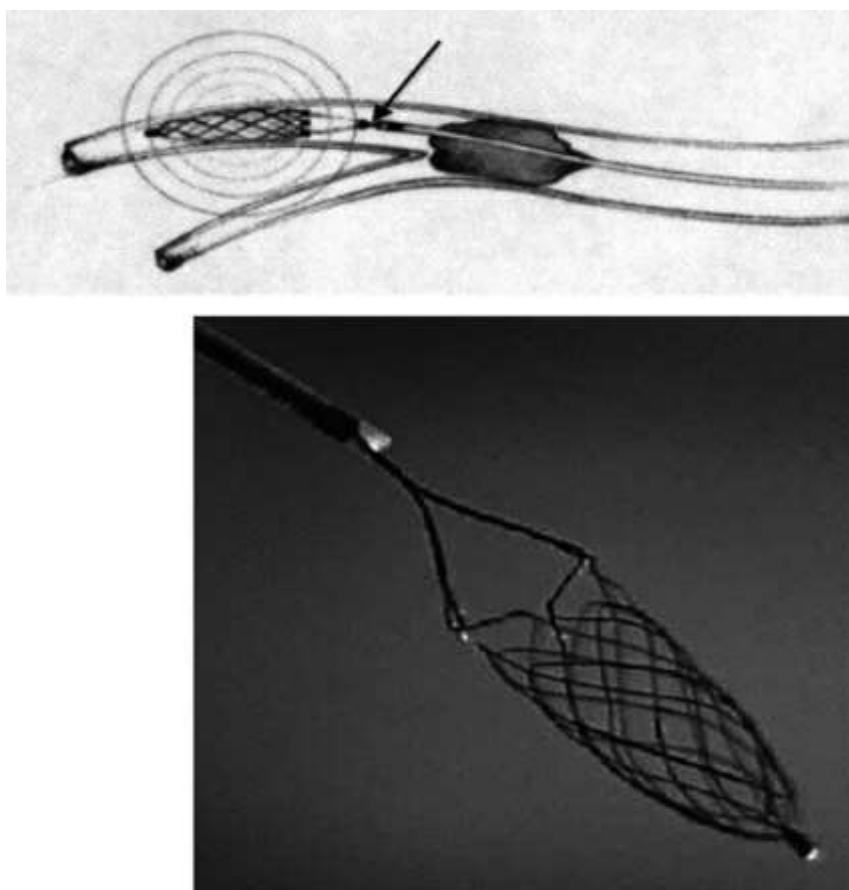


Figure 6. Catch device® (Balt Extrusion, Montmorency, France).

Phenox Clot Retriever® (Phenox GmbH, Bochum, Germany)

This Germany-based company offers a whole range of unique-design mechanical thrombectomy systems.

Phenox clot retriever - pCR® is a device with a brush-like structure—Figure 7. The fibre structure made from nylon (polyamide) filaments increases in diameter from proximal to distal end. The fibres have also X-ray markers at the both distal and proximal ends. The fibre work, which has large surface area for efficient thrombus removal, is connected to a three-meter-long guiding wire, which is introduced into the body through a micro-catheter. This system is 22 millimetres long and is available in three different diameters intended to treat vessels ranging in diameter from 1 to above 3 millimeters. The pCR is introduced through microcatheter. Upon deployment behind the occluding thrombus, the filaments unfold, and the thrombus is captured in the fibre network. The system is then gradually withdrawn into the guiding catheter under constant aspiration and removed.

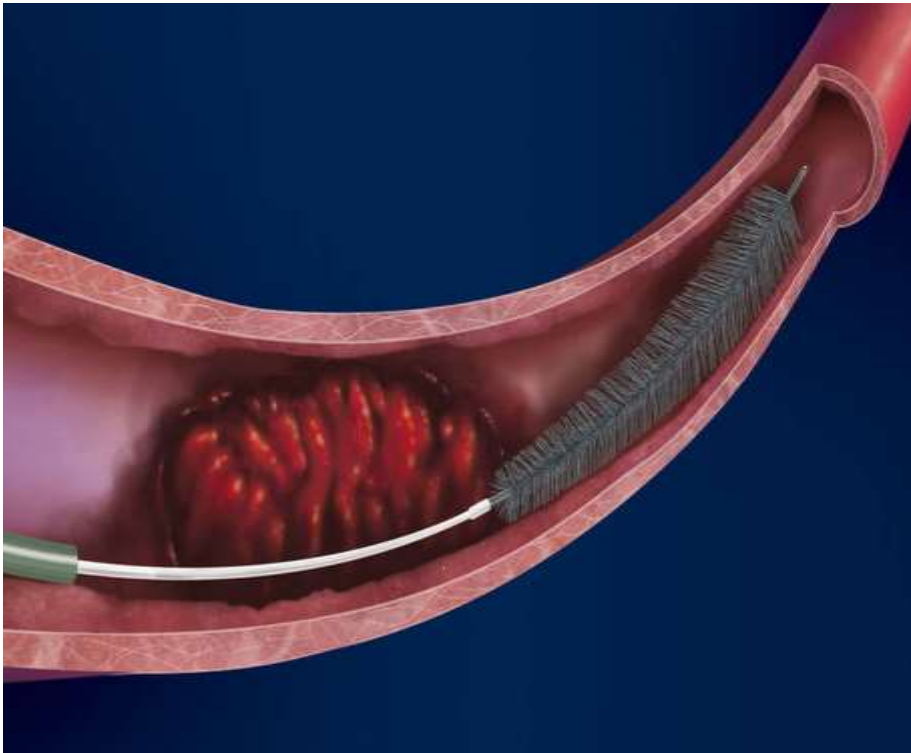


Figure 7. Phenox clot retriever - pCR® (Phenox GmbH, Bochum, Germany).

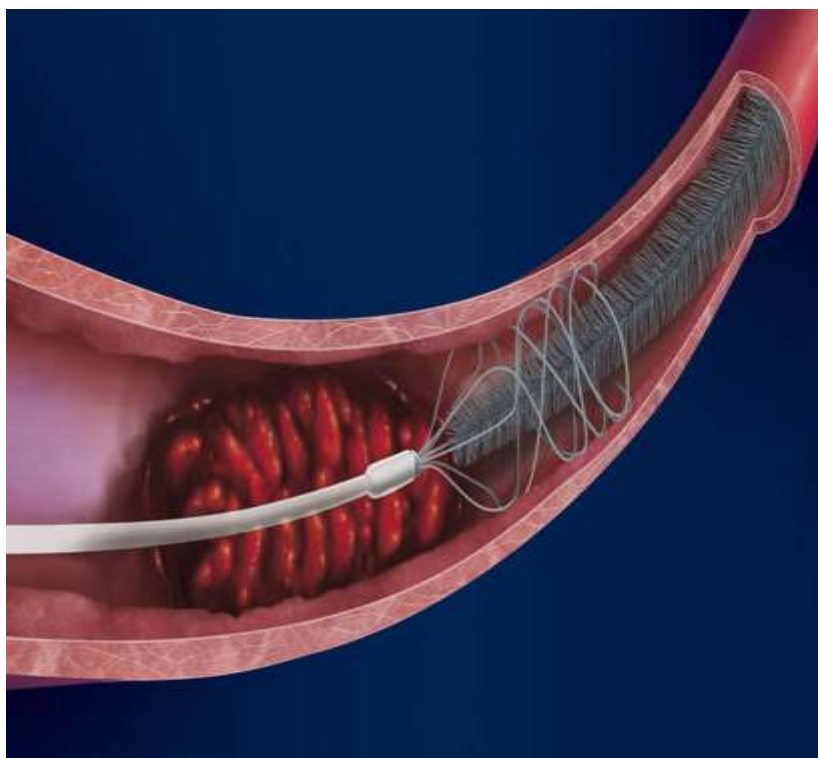


Figure 8. CRC phenox Clot Retriever CAGE® (Phenox GmbH, Bochum, Germany).

CRC phenox Clot Retriever CAGE®, like the pCR, is made from a fibre work of polyamide filaments. In contrast to the pCR, the CRC possesses an additional Nitinol (nickel, titanium) thread cage at the proximal end of its fibre brush—Figure 8. This nitinol cage gives a higher radial range, which is an advantage against older and/or firmer thrombi. This system is available in two different sizes intended to treat vessels with diameter ranging from 2 to above 3 millimetres. Otherwise, the design of the polyamide fibres, central wire, insertion wire and system markers is identical to the pCR system. The system insertion, deployment and final thrombus removal is also performed in the same way.

Both the pCR and the CRC systems are intended for single extraction only, which means that they must be exchanged for different devices in case of first attempt extraction failure.

Bonnet Intracranial Flow Restoration Device® (Phenox GmbH, Bochum, Germany)

The BONNET Intracranial Flow Restoration Device® consists of a self-expanding nitinol braiding with polyamide filaments passing through the interior—Figure 9. These enlarge the surface area and enable better fixation of the thrombus mass. The instrument is firmly connected with an insertion wire, and carries X-ray visible markers at its proximal and distal ends. The size of this device is 5 x 35 mm.

The BONNET® has high flexibility and is easy to advance through tortuous vessels. It is also a repeatedly re-usable device. The system is advanced through microcatheter and should

preferably be deployed distally of the thrombus. However, it can also be released after withdrawal of the micro-catheter partially or completely into the thrombus. The micro-catheter does not have to be completely removed. The flow restoration is achieved through simultaneous withdrawal of the BONNET® and the micro-catheter through the guiding catheter. The system can either be put distally of the thrombus or can be released into the thrombus. Re-use in the same patient is possible, for example in another vessel blockage.

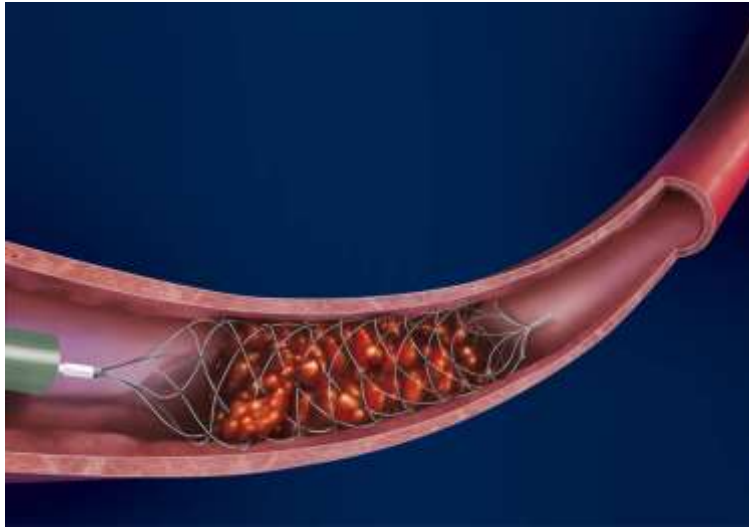


Figure 9. BONNET Intracranial Flow Restoration Device® (Phenox GmbH, Bochum, Germany).

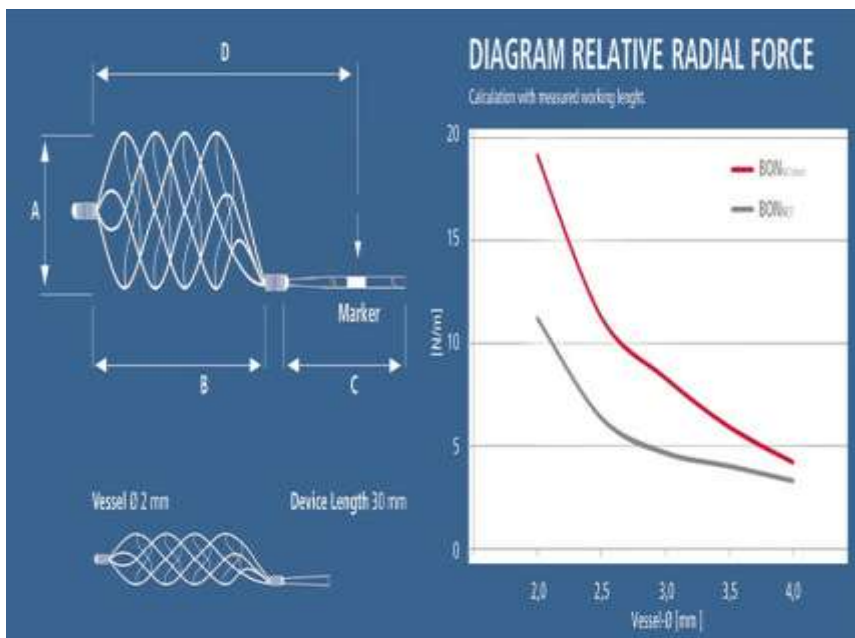


Figure 10. BONNET short Intracranial Flow Restoration Device® (Phenox GmbH, Bochum, Germany).

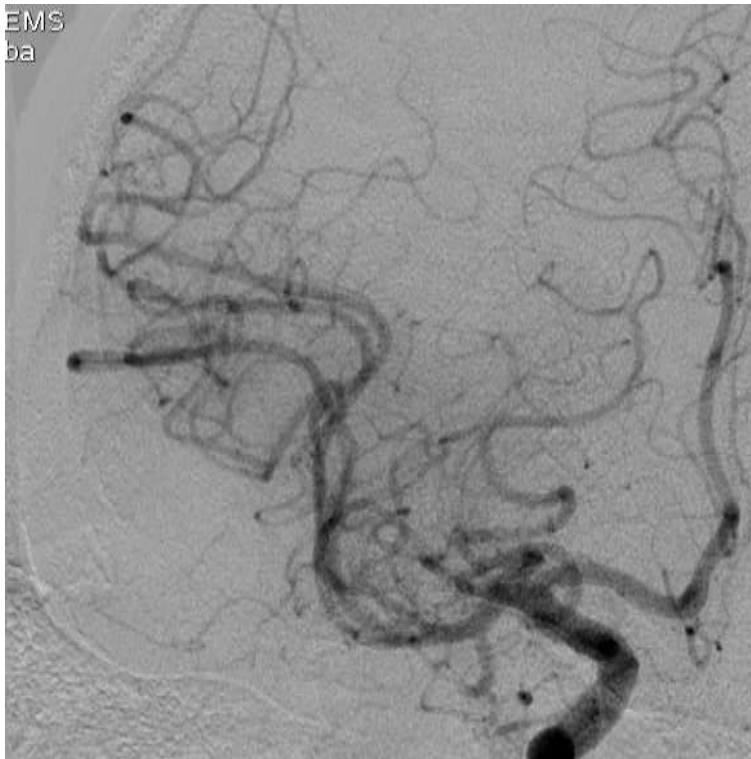
The BONNET short Intracranial Flow Restoration Device® is a new variant of the trusted BONNET®. In addition to its new size, the system displays a new shape and a higher level of relative radial force. The BONNET short® consists of a self-expanding nitinol braiding with polyamide filaments passing through the interior—Figure 10. The system is firmly connected with an insertion wire in an eccentric attachment and carries X-ray visible markers at its proximal and distal ends. It enables a fast and safe intracranial vessel flow restoration also in curved vessel areas. Re-use in the same patient is possible, for example in another vessel blockage. Its size is 5 x 23 mm, and it is suitable for vessels larger than 2 mm. The technique of its deployment and withdrawal is the same as in BONNET system®.

CASE REPORT 2

A 48-year-old male suffered from sudden left-sided hemiparesis, left facial nerve palsy and dysarthria (NIHSS 10 points). Findings on brain CT six hours after stroke onset were negative; CTA detected M1-MCA occlusion. CT perfusion did not detect any signs of definitive cerebral infarction. Digital subtractional angiography confirmed right MCA occlusion with collateral flow from the ACA territory (Figure 11a). Mechanical thrombectomy with BONNET device was performed 40 min after admission to hospital and immediate recanalization of MCA was achieved (Figure 11b). Patient's clinical status substantially improved immediately after the vessel reopening. Patient was without neurological deficit (mRS 0) at discharge.



Figures 11 Continued



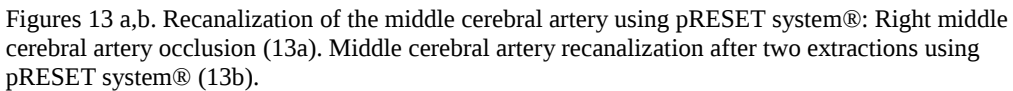
Figures 11 a,b. Recanalization of the middle cerebral artery using BONNET system®: Complete right middle cerebral artery occlusion with collateral flow from the anterior cerebral artery territory (11a). Successful middle cerebral artery recanalization using the BONNET system® (11b).

pRESET Thrombectomy Retriever® (Phenox GmbH, Bochum, Germany)

The pRESET Thrombectomy Retriever® is another brand new thrombectomy device. The laser-cut and self-expanding Nitinol-System allows effective removal of clot material from 2 to 4 mm vessel diameter. An optimized cell design and a helically shaped slit enable a constant cell size, even in smaller vessels. The closed “ring” design at the proximal end of the system ensures a stable opening, less tapering when withdrawn and radial force on a steady level—Figure 12. The pRESET features an excentric attachment to the insertion wire as well as X-ray visible markers at its proximal and distal ends. The System can be cleaned easily, and re-use in the same patient is possible. Its nominal size is 4 x 30 millimetres; it is delivered through 0.021” lumen microcatheter, and the technique of thrombus extraction is identical with previously described systems.

CASE REPORT 3

A 51-year-old male was admitted to the hospital with sudden left-sided hemiparesis and dysarthria (NIHSS 16 points). Chronic abuse of alcohol and nicotine were in patient history.



The recanalization rates clinical results of modern thrombectomy devices appear promising as the devices continue to evolve and become more operator-friendly. Baker et al. [34] screened citations for human studies of any design or case series or case reports of patients with an acute IS that evaluated a neurothrombectomy device and reported at least one clinical effectiveness outcome or harm. They identified 87 articles meeting eligibility criteria: 18 prospective single-group studies, seven noncomparative retrospective studies, and 62 case series or case reports. All prospective and retrospective studies provided data on successful recanalization. The recanalization rate widely varied (43 to 78% with the MERCI Retriever and 83 to 100% with the Penumbra System). SICH was evaluated in 16 studies and did not exceed 11%, and vessel perforation or dissection was between 0 and 7% and varied by device. Predictors of harm included older age, history of stroke, and higher baseline stroke severity scores, whereas successful recanalization was the sole predictor of good outcomes [34]. Unfortunately, the limitation of performed studies is missing evidence of a fragmentation, distal embolization, or relocation of the occlusive thrombus [35].

Three studies are underway in the United States and Europe to test retrievable stents in the treatment of acute IS. The Solitaire™ FR with the Intention for Thrombectomy (SWIFT) study is ongoing (recruitment completed) and will compare Solitaire FR with the Merci retriever in 200 patients. [36] The Trial and Cost Effectiveness Evaluation of IA Thrombectomy in acute IS (THRACE) study that will compare IVT with thrombectomy procedures using the Merci retriever, Penumbra system, Catch device (Balt Extrusion,

Montmorency, France), and the Solitaire™ FB stent in 480 patients. The Thrombectomy Revascularization of Large Vessel Occlusions (TREVO 2) study was recently started in the United States and will compare the Trevo system versus the Merci retriever in the treatment of acute IS. Approximately 178 patients will be enrolled in the study [37].

The aim of an ongoing IMS-III trial is to investigate whether a combination of IVT and IAT is superior to standard IVT (standard dose of 0.9 mg/kg rt-PA). If the thrombus is resolved, no additional intra-arterial therapy is administered. If the occlusion is still present, the additional intra-arterial treatment (max. 22 mg of rt-PA) will be administered. IAT might be combined with low-intensity ultrasound (US) via the EKOS® Micro-Infusion Catheter and thrombus-removal device as well. The clinical outcome is calculated by the mRS three months after the procedure. A total of 900 subjects will be enrolled in the study [38].

The ongoing Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR-RESCUE) trial aims to evaluate the efficacy of the standard IVT in case of proximal main branch occlusion in the anterior circulation compared to the efficacy of mechanical thrombectomy (with MERCI or Penumbra) within eight hours after stroke onset [39].

In the DWI/PWI and CT perfusion assessment in the triage of wake-up and late presenting strokes undergoing neurointervention (DAWN) trial, patients with large artery occlusion are selected according to the magnetic resonance imaging (MRI) or CT perfusion images more than eight hours after stroke onset. The treatment is initiated between eight and 24 hours [40].

Direct Stent Placement

Intracranial self-expanding microstents have been used successfully as temporary or permanent endovascular bypass in the invasive management of acute IS. It has been performed most commonly as a rescue procedure to displace thrombus or following early reocclusion after successful fibrinolysis. Angioplasty with stenting was originally described in acute stroke for underlying atherosclerotic stenosis after successful fibrinolysis.

With this technique, the stent deployed at the point of vessel occlusion expands, displacing circumferentially the thrombus and allows flow restoration. The re-establishment of flow through the occlusion site promotes immediate dissipation of prothrombotic factors. At the same time, the restored arterial flow allows thrombolytic medications to be in contact with thrombotic material trapped within the mesh of the stent and against the arterial wall.

Various stents have been used over time to bridge the occlusion site. The first report of coronary balloon-mounted stent placement into the basilar artery was published in 1999, by Phatouros et al. [41]. These procedures were associated with high periprocedural major complication rates (up to 50%). Gradually, self-expanding stents came into use. In the past, stenting was used after failure of different recanalization approaches, i.e., angioplasty, IAT and thrombectomy with a snare device.

Self-expandable stents were approved for vessel remodeling in the treatment of cerebral aneurysms and intracranial atherosclerotic disease. The Enterprise stent (Cordis Neurovascular, Miami Lakes, FL, U.S.), the Neuroform system (Stryker, Natick, MA, U.S.), and the Leo stent (Balt Extrusion, Montmorency, France) are devices introduced for stent-assisted coiling of wide-neck aneurysms. The latter stent is not marketed in the United States.

The Wingspan system (Stryker, Natick, MA, U.S.) was approved for treatment of intracranial atherosclerosis. Both the Neuroform and Wingspan stents have an open-cell design, whereas the Enterprise and Leo stents are closed-cell design—Figure 14. [42] The closed-cell design allows resheathing of the stent after partial deployment (70% for Enterprise and 90% for Leo).

These devices have been used off-label in the treatment of acute IS. The concept of flow restoration without clot removal has gained attention as other recanalization approaches that focus their strategy in thrombectomy have failed.

Published Studies

Fitzsimmons and Nelson reported one of the first cases of cerebral revascularization with deployment of a self-expansible stent. [43] A Neuroform stent was deployed in an occluded left MCA after the IA infusion of a glycoprotein (GP) IIb/IIIa inhibitor failed to recanalize the vessel. Multiple reports have followed, especially from the group lead by Levy et al. [44], who have pioneered the use of self-expansible stents in the treatment of acute IS. In 2007, they reported a 100% successful deployment of stents (16 Neuroform and three Wingspan stents) in the target occlusion, achieving a recanalization TIMI score 2 – 3 in 79% of lesions. Four (22%) patients had a mRS ≤ 3 at three months and two (11%) patients suffered SICH.

Most of the studies published to date have been retrospective single-center reports with sample sizes ranging between nine and 20 patients [45–49]. Although these cohorts are small, recanalization rates of 92 and 100% have been reported in cases where other thrombectomy devices have failed [46,47,49]. Another two small studies reported recanalization rates with cerebral angioplasty 60 to 90% and good outcomes (mRS 0 – 2 or NIHSS ≤ 4) between 36 and 74% [50,51]. Jovin reported successful recanalization in 23 out of the 25 patients, one SICH and one carotid dissection. [52]

The recently reported preliminary data of a prospective Stent-Assisted Recanalization in acute IS (SARIS) study of first 20 patients with acute IS showed the successful recanalization (TIMI grade 2/3) in 100% of cases with a 5% rate of SICH. [53] A six-month follow-up of the SARIS trial found mRS ≤ 2 in 55% of patients with a mortality rate of 35%. None of the patients had in-stent restenosis and all had TIMI-3 flow in angiographic follow-up [54].

There is growing experience with intracranial stenting in the acute IS setting, which has brought encouraging findings. Stenting is technically feasible, as reported up to 100% successful deployment rate of different studies. Acute in-stent thrombosis is rare, and different treatment strategies may be used to achieve stent recanalization: intra-arterial administration of GP IIb/IIIa inhibitors or stent resheathing. Enterprise stents appear to have better navigability than the Wingspan and Neuroform stents, especially in acute IS patients who generally have a tortuous anatomy with atherosclerotic changes. Self-expanding stents have enough radial force to recanalize occluded arteries. Angiographic outcomes have improved since the first series published by Levy et al. achieving TIMI 2/3 flow in more than 90% of lesions. Antiplatelet agents such as aspirin and clopidogrel or GP IIb/IIIa inhibitors should be administered prior to stent deployment to avoid acute in-stent thrombosis. However, this may lead to SICH if thrombolytic agents have been previously administered.

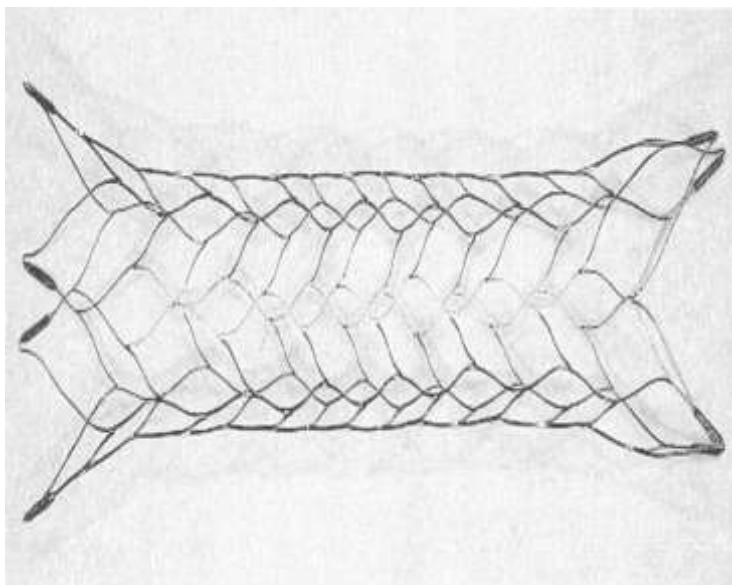


Figure 14. Enterprise stent (Cordis Neurovascular, Miami Lakes, FL, U.S.).

Intracranial stenting in acute IS patients certainly has many potential drawbacks. Permanent stent deployment in the setting of acute IS requires platelet inhibition to reduce the possibility of in-stent thrombosis. Intra-procedural administration of GP IIb/IIIa inhibitors or inhibitors of platelet function (Anopyrin®, Cardegic®) may increase the risk of hemorrhagic transformation. Furthermore, dual antiplatelet therapy is continued for at least the initial three months after the procedure. This is particularly worrisome in patients who already have been treated with IVT and/or IAT. Stent placement in acute IS is a permanent implant to resolve a temporary occlusion that may be caused by an embolus. Stent deployment may over time result in delayed in-stent stenosis. With expansion of the stent, the thrombus material may be pushed into perforating arteries. This phenomenon, described as “snow-plowing,” may explain strokes in perforator territories once the main artery has been recanalized and may pose another drawback of this technique.

CASE REPORT 4

A 61-year-old female suffered from sudden loss of consciousness, dysarthria and right-sided hemiparesis (NIHSS 15 points). Only hypercholesterolemia was in medical history. CTA revealed the occlusion of the left M1-MCA. Patient was treated with IVT (80 mg of rt-PA) 115 minutes after stroke onset without improvement of the clinical status. Persistent occlusion of the MCA was detected on angiography (Figure 15a). Left MCA was recanalized using cerebral percutaneous angioplasty with intracranial stent implantation three hours and 50 minutes after symptoms onset (Figure 15b). Follow-up brain CT detected infarction in the left basal ganglia. On discharge, patient presented with severe right-sided central hemiparesis and global aphasia (mRS 5).



Figures 15 a,b. Recanalization of the distal occlusion of the internal carotid artery and the middle cerebral artery using cerebral percutaneous angioplasty and stenting: Distal occlusion of the internal carotid artery demonstrated on angiography (15a). Recanalization of the internal carotid artery and the middle cerebral artery after direct intracranial stent deployment (15b).

CASE REPORT 5

A 77-year-old male suffered from vertigo and vomiting, gradual progression of dysarthria and left-sided hemiparesis. Arterial hypertension and diabetes mellitus were in medical history. Severe basilar stenosis was detected on CTA performed two hours and 50 minutes after stroke onset. Upon transfer to angiography, tachypnoe, worsening of consciousness and quadriparesis evolved. Patient was intubated and angiography detected left vertebral artery stenosis and basilar artery occlusion (Figure 16a). Attempt to recanalize the occlusion was

undertaken 3.5 hours after onset of symptoms. However, the procedure failed with contrast media paravasation at the site of vertebral intradural segment dissection caused by microwire (Figure 16b). Follow-up CT scan revealed ischemic changes in the pons and cerebellum and subarachnoid bleeding in posterior fossa. Patient was discharged in a poor clinical state (mRS 5).



Figures 16 a,b. Complications during recanalization of the basilar artery occlusion: Diffuse left vertebral artery atherosclerotic lesions with severe stenosis of V3-segment of left vertebral artery and artery occlusion distal to posterior inferior cerebellar artery origin (16a). Severe paravasation after iatrogenic vertebral dissection caused by microwire passage (16b).

Endovascular Sono-Lysis—EkoSonic™ Endovascular System (EKOS Corporation, Bothell, WA, U.S.)

Although the complex effect of ultrasound on the acceleration of thrombus lysis is not yet fully understood, it is assumed that the ultrasonic waves accelerate enzymatic fibrinolysis by primarily non-thermal mechanisms—increasing the transport of fibrinolytic agents into the thrombus by mechanical disruption of its structure [55], direct activation of fibrinolytic enzymes, either mechanical breaking of the complex molecules, in which fibrinolytic enzymes are inactivated by binding to their inhibitors, or irritation of the endothelium with increased production of fibrinolytic enzymes [56,57], transient peripheral (capillary) vasodilatation caused probably by increased production of nitrite oxide in the endothelium [58,59]. Radiation force and acoustic cavitation are the next possible and discussed mechanical effects of ultrasound [60].

There are two possibilities for sono-lysis application—transcranial approach through intact skull or intravascular approach.

The EkoSonic™ Endovascular System (EKOS Corporation, Bothell, WA, U.S.) is intended for targeted local drug delivery including rt-PA where non-cavitation, high-frequency, low-power microsonic energy allows better penetration and attachment of a thrombolytic agent into the thrombus, resulting in faster lysis and a vessel recanalization, as was proved by manufacturer in in vitro testing—Figure 17 a,b. The system consists of a single-use EKOS MicroLys US infusion catheter intended either for neurovascular interventions EkoSonicSV™ (3F/150 cm catheter inserted over a 0.014" wire) or for peripheral procedures EkoSonic™ (over a 0.035" wire). The outer diameter of the catheter is rather small and can be easily introduced through 6F guiding catheter, which is advantageous compared to some other devices. The catheters have a hollow end-hole infusion lumen incorporating cylindrical piezo transducer at its tip, which converts high-frequency energy into ultrasound waves. In EkoSonicSV™, the transducer power output is 0.45 W at a frequency of 1.4 – 1.9 MHz. The energy deployment causes non-cavitation fibrin strands thinning, loosens up the fibrin matrix and thus increases the availability of plasminogen activation receptor sites for plasminogen/plasmin binding. Bound plasminogen/plasmin is then protected from α_2 -antiplasmin inhibition. According to the manufacturer's data, thrombus exposed to ultrasonic pressure absorbed 48% more rt-PA in one hour than in conventional catheter local drug delivery. The clot lysis is four times faster than with the conventional catheter-directed thrombolysis alone in manufacturer in-vitro experiments. It has been suggested that EkoSonicSV™ dissolves a clot more homogeneously and completely with minimal residual thrombi and a low risk of distal embolism by dislocated clot fragments. There is a thermoreceptor placed at the catheter tip for continuous intraprocedural temperature monitoring. Increase in temperature may indicate vessel patency or local accumulation of energy in the clot and surrounding tissues by ultrasound waves. Since the output power and catheter temperature are continuously monitored, this therapy poses a low risk of the vessel wall and surrounding tissue damage and impairment. Due to these reasons, the vessel patency is reached faster with the smaller total doses of thrombolytic agents when compared to standard thrombolysis.

The other part of the system is the central unit, which generates the specific frequency energy and monitors the output power, along with the temperature of the catheter tip—Figure 17 a,b.

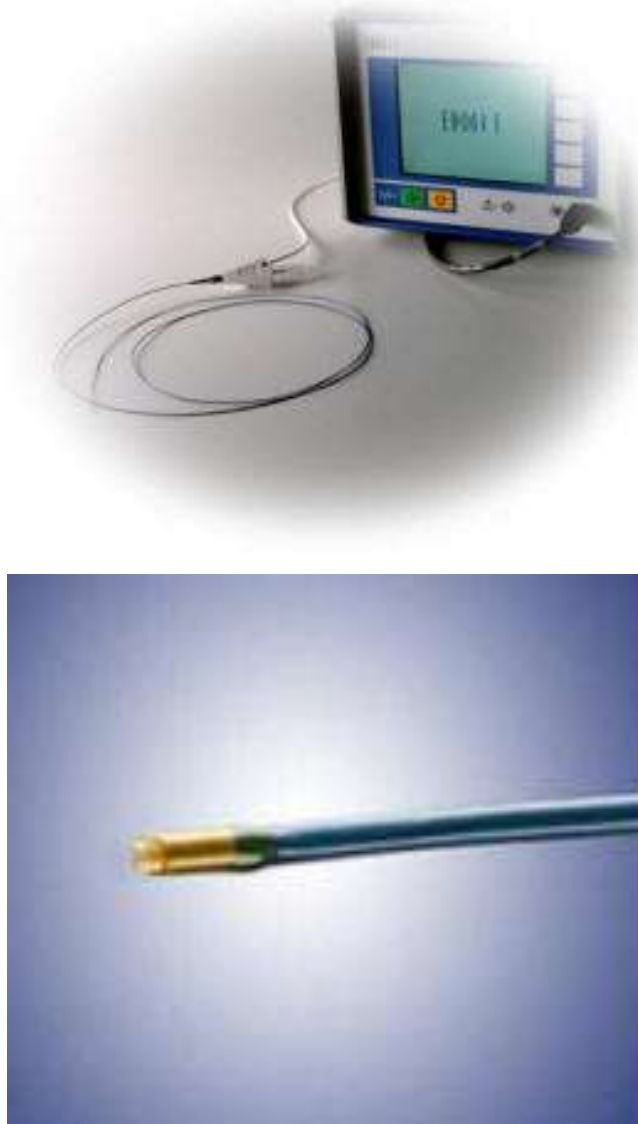


Figure 17 a,b. EkoSonic™ Endovascular System (EKOS Corporation, Bothell, WA, U.S.).

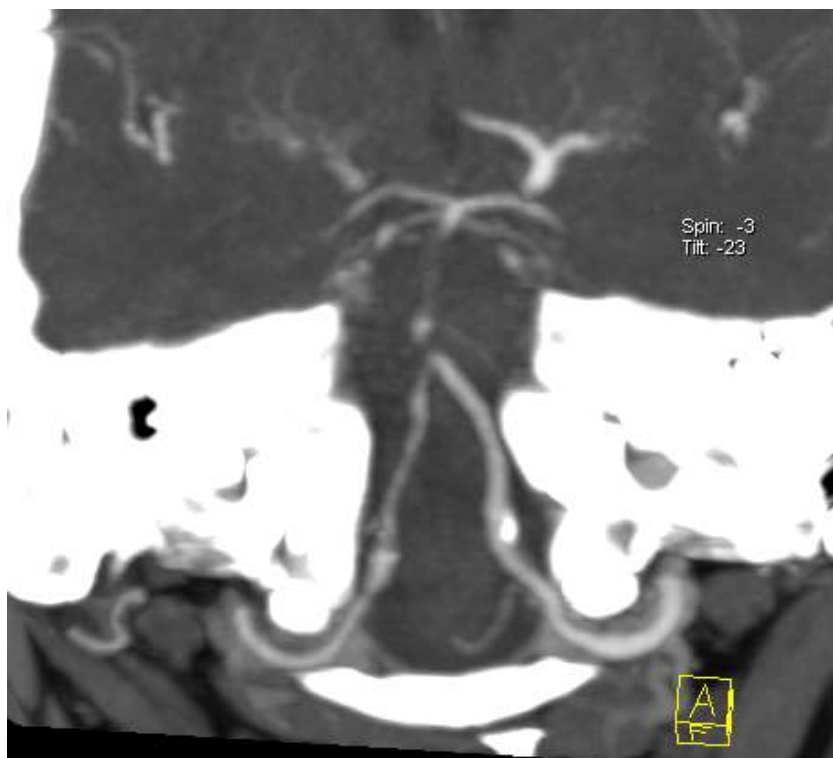
Published Studies

A phase I trial reported preliminary results of treatment using the EkoSonicSV™ system in 14 patients with 60% recanalization at 60 minutes, despite treatment of large clot burdens in some patients [61]. The device was tested again in the Interventional Management of Stroke (IMS) II with promising results [62]. Complete recanalization was achieved within 60 and 120 minutes in 12 (41%) and 20 (68.9%) patients, respectively. The authors compared their results with the findings from the IMS-1 clinical trial. They concluded that recanalization has been achieved in seven (30.4%) of the 23 control patients from the IMS-1 trial. Pooled analysis based on the results of these two studies showed complete recanalization

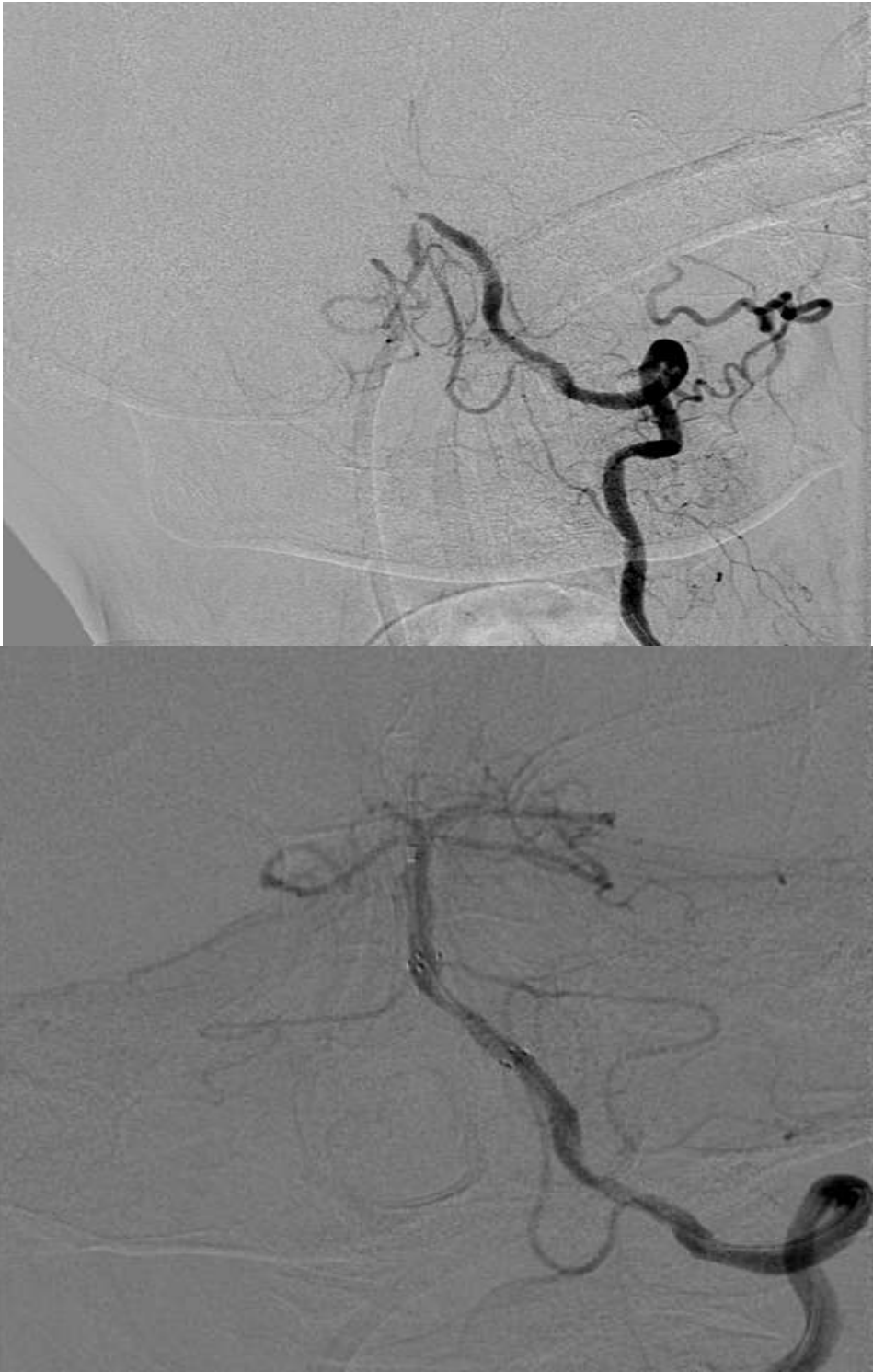
in 120 minutes in 68.9% of patients in the EkoSonic™ catheter sonography group compared with 53.3% of patients treated by standard microcatheter infusion technique. Due to these promising results, acute stroke in NINDS rt-PA-funded IMS III trial globally, which is currently enrolling at 60 stroke centers worldwide [38]. Furthermore, the SICH occurred in 8/81 (9.9%) subjects in the IMS-II, including 1/26 (3.8%) patients treated with IV rt-PA alone and one in a sonography microcatheter-treated subject without the use of sonography. No direct vessel perforation, primary subarachnoid hemorrhage, or intracranial dissection was documented in the treated subjects.

According to the authors' experiences, the EkoSonic™ catheter is easy to introduce despite the non-flexible sonography transducer located at its distal tip [63]. The authors did not encounter any detectable distal embolization potentially resulting from device manipulation. However, the risk of distal embolization was an issue in Janjua's study [64]. Although the distal emboli were observed in their study, this situation did not have any significant clinical consequences. On the other hand, distal embolisation was not detected in other studies [65,66]. The clot resolution time in patients presented by the authors (vessel patency in 35 minutes) was much quicker than in the IMS-2 study, where complete resolution of a clot was achieved after one hour in 41.1% of patients. One may speculate that such a promising result may be caused by the fresh (noncrossed-linked) nature of the clot.

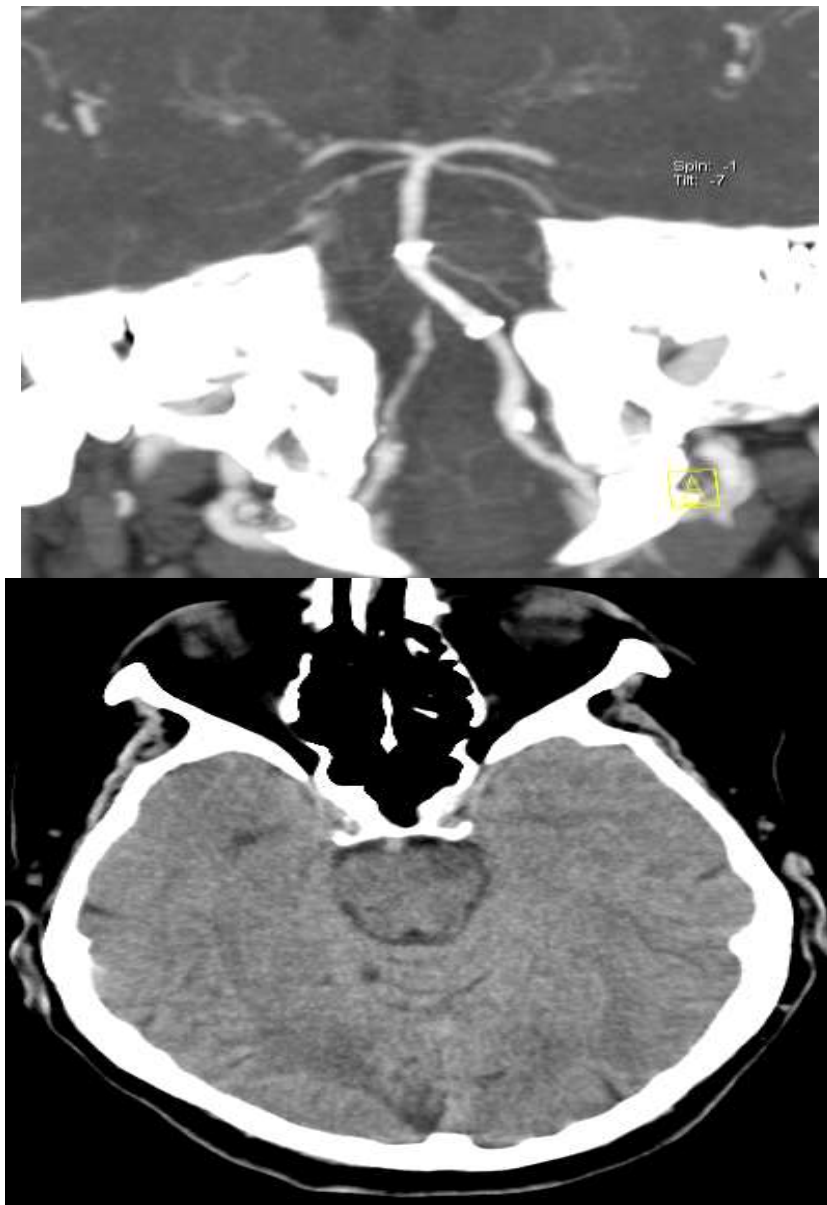
CASE REPORT 6



Figures 18a-e. Continued on next page



Figures 18a-e. Continued on next page



Figures 18a-e. Recanalization of the basilar artery using intravascular sonolysis by EkoSonicTM Endovascular System. CT angiography detected severe proximal basilar artery stenosis in junction of vertebral arteries and occlusion of distal part of basilar artery; the tip of basilar artery and posterior cerebral arteries are perfused through the posterior communicating arteries collateral blood flow (18a). Angiographic finding of basilar artery occlusion via the left vertebral artery (18b). Cerebral percutaneous transluminal angioplasty with Wingspan stent 3,5 x 15 insertion to the proximal basilar artery stenosis and EkoSonicTM microcatheter sonolysis 35 minutes after rt-PA administration (18c). Follow-up CT angiography (maximum intensity projection—MIP) showed patent basilar artery with stent and normal filling of bilateral anterior inferior cerebellar artery, superior cerebellar artery and posterior cerebellar artery. Severe stenosis of the right vertebral artery is apparent (18d). CT scan at day two showed infarctions in the left pons and in the right posterior cerebral artery territory - calcarine artery feeding territory (18e).

A 60-year-old female was admitted to a local hospital with right humeral neck fracture. Four hours later, while being on a conservative therapy regime, she was suddenly unable to speak, and right-sided hemiplegia was diagnosed (NIHSS 19 points). Arterial hypertension, hypercholesterolemia, ischemic heart disease with coronary bypass and diabetes mellitus were in medical history. The immediate CT scan was negative for signs of ischemia and haemorrhage. CTA showed severe stenosis of both vertebral arteries at the junction level and basilar artery occlusion (Figure 18a). CT perfusion scans revealed prolonged time to peak value in the posterior fossa structures and occipital lobes of the brain. No substantial changes in blood volume and blood flow parameters were observed. The patient was transferred to angiography, which confirmed findings of basilar artery occlusion via the left vertebral artery. Intracranial stent Wingspan 3.5/20 mm (Boston Scientific, Fremont, CA, USA) was placed into the proximal basilar artery stenosis (Figure 18c). Thereafter, the EkoSonic™ catheter insonation was performed (Figure 18c) and completed successful basilar artery recanalization eight hours after stroke onset was achieved (Figure 18d). Follow-up CT revealed small brainstem infarction (Figure 18e). At discharge, residual dysarthria and right-sided central hemiparesis persisted (mRS 4).

Other Experimental Devices EPAR®—Endovascular Photoacoustic Recanalization (Endovasix Inc., Belmont, CA, U.S.)

EPAR® is a mechanical clot fragmentation device based on laser technology (endovascular photo-acoustic reanalization—EPAR®). This device consists of three French hydrophilically coated microcatheters with five windows at its tip, each of them with one optic fiber. The photonic energy, delivered at a rate of 1,000 optic pulses per second, is converted into acoustic energy at the fiberoptic tip of the catheter. This in turn creates micro-cavitation bubbles. The cavitation pockets undergo expansion and subsequent collapse and should create suction of the thrombus through the lateral windows into the catheter tip. Inside the tip, the clot is emulsified, and the tip contents are discharged as subcapillary-size particles (in vitro: 1 to 10 µm). This system is intended as fast clot emulsifier rather than laser-ablation device. The main objective is to provoke mechanical thrombolysis and reduce the amount of pharmacological thrombolytics needed.

Studies

Thirty-four patients (median NIHSS 19) were enrolled in the study published by Berlis et al. [67]. Ten patients had ICA occlusion, 12 patients MCA occlusion, 11 patients vertebrobasilar occlusion, and one patient posterior cerebral artery occlusion. The overall recanalization rate was 41.1%. Complete EPAR® treatment was possible in 18 patients (median NIHSS 18), with vessel recanalization achieved in 11 patients (61.1%) after EPAR. The average time of procedure was 9.65 minutes. Incomplete EPAR® treatment (16/34, median NIHSS 19) was defined as intention to treat with EPAR® and that the EPAR® microcatheter entered the patient. Additional treatment with IA application of rt-PA was used in 13 patients. An adverse event associated with the use of the device occurred in one patient. SICH occurred in two patients (5.9%). The mortality rate was 38.2%. Unfortunately, this device is not marketed any longer.

Revive™ SE Blood Clot Retrieval® (Codman and Shurtleff, Inc., Johnson and Johnson Company, Raynham, MA, U.S.)

Revive™ SE® is intended as a blood clot retrieval and removal device. Its self-expanding nitinol basket should act as a temporary bypass across the occlusion and subsequently can be used for thrombus removal. It is introduced via a microcatheter directly into or beyond the lesion and can be redeployed several times. It is designed as a closed distal end basket that should engage the clot effectively and prevent it from distal embolisation.

The company received CE Mark approval for the Revive™ SE® in February 2011. The device is not approved for distribution in the United States.

Pilot results of ten patients with acute large vessel occlusions treated with the Revive device was published by Rohde et al. Vessel recanalization (TICI 2b or 3) was successful in all patients without device-related complications. After one month, six patients had a clinical improvement of >8 points or an NIHSS 0 – 1; three patients died. SICH occurred in two patients. [68]

This device is going to be investigated in a single-arm German study named RIVER I (Reperfuse Ischemic Vessels with Endovascular Recanalization). The patients will be screened before the procedure with MRI imaging.

Penumbra Separator™ 3d® (Penumbra, Alameda, CA, U.S.)

Penumbra Separator™ 3D® is a brand new product that was introduced in January 2012, dedicated for thrombus retrieval in acute stroke patients. Its design resembles complex 3D “basket.” Its inner struts and complex design is aimed at firm thrombus engagement and reliable retraction. Like other stentriever, it is deployed into the occluded vessel segment with micocatheter, and retrieval is bolstered by active aspiration via guiding catheter.

At ABC-WIN Seminar, Prof. M. Knauth presented the European evaluation data on Separator 3D [69]. The first preliminary data from six European centers with this new device showed an approximately 90% successful recanalizations and good deliverability even in tortuous vessels.

Mindframe Capture LP® (Mindframe Inc., Irvine, CA, U.S.)

MindFrame capture LP® is another stent-like device aimed at temporary by-pass reopening of the occluded vessels and effective thrombectomy—Figure 19.

Its advantage is compatibility with 10/14 microcatheters with inner diameter from 0.0165 inch. It received an CE mark in August 2011, and first results in treating stroke patients were presented by Tommy Andersson, MD, PhD, Department of Neuroradiology, Karolinska University Hospital, Stockholm, who stated that this devices allowed good ability to access thrombus through tortuous blood vessel anatomies and showed fair recanalizations rate (personal communications).

Acandis APERIO® System (Acandis GmbH and Co. KG, Pforzheim, Germany)

Acandis APERIO® System is a self-expanding stent-like clot retrieval system that can be deployed through 0.027" inner lumen microcatheter and bolsters superb trackability and good thrombus removal rate of about 80%—Figure 20. No published data are available so far.

Possis Angiojet System® (Possis Medical, Minneapolis, MN, U.S.)

This is a five-Frech system based on rheolytic thrombectomy effect, caused by fast high-pressure saline jets that creates distal Venturi suction and leads to clot fragmentation and aspiration. First case reports of ICA recanalization procedures with this device were published [70].



Figure 19. Mindframe capture LP®.

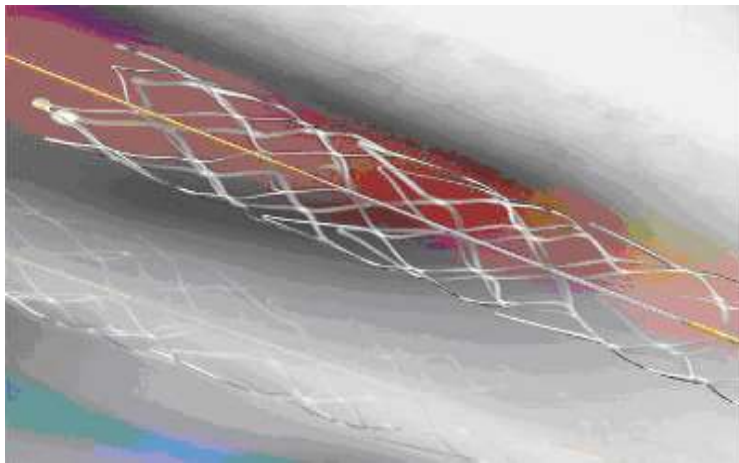


Figure 20. Acandis APERIO System®.

Latis Laser Device® (Spectanetics, Colorado Springs, CO, U.S.)

This is also a microcatheter with laser at its tip. Contrast media injection was used for energy transfer from the catheter into the thrombus. The safety and feasibility study was

stopped after enrollment of only 12 patients, since there were difficulties in deployment of the catheter to the level of occlusion. This device is not marketed any longer.

Amplatz Goose-Neck Microsnare® (Ev3 Medical, Plymouth, MN, U.S.)

The system consists of microcatheter, snare and rotator. The microsnare is constructed of a nitinol cable and gold plated tungsten loop. This nitinol snare was used for clot retrieval in cases of solid thrombus with firm consistency. In cases of soft thrombus, multiple passes of the snare through the thrombus were used to disrupt its structure.

Attractor-18™ (Boston Scientific/Target Therapeutics, Fremont, CA, U.S.)

Another so called “goose-neck” snare, primarily developed to retrieve dislodged coils during cerebral aneurysm coiling procedure. Both these devices were not originally intended for thrombectomy in IS patients.

Neuronet™ Guidant Device® (Guidant Corp, Temecula, CA, U.S.)

Neuronet is a self-expanding basket introduced distally to a clot by a microcatheter. After it unfolds, it is retracted back into the thrombus and should pull the thrombus into the micro catheter.

In-Time™ Retrieval Device® (Boston Scientific, Boston, MA, U.S.)

The principle of clot removal is the same as in the Neuronet Guidant device.

Interventional Procedure Technique

In the authors' department, most of the procedures are performed under conscious sedation or under general anesthesia. Common femoral artery is the most common access site. In a few patients, in whom iliac arteries were occluded, the recanalization procedure was performed via brachial access. Mostly 6F or 8F sheath and 4F diagnostic catheters introduced over hydrophilic wires are used. As the first step, brain supplying vessels angiography is performed to evaluate the site of occlusion and extent of collateral supply. When endovascular recanalization is indicated, the 6F or 8F guiding catheter is inserted into the ICA or the vertebral artery. Subsequently, microcatheter supported by microwire sized 0,010” to 0,018” is inserted into the occlusion site. The authors try to navigate the microcatheter past the occlusion site to verify the lengths of the occlusion and to check if distal embolization is present. It is an absolutely duty to perform control microcatheter angiography after passing the occlusion to confirm the intraluminal position of the catheter and to exclude any vessel dissection or perforation. Having done this, the decision as to which of the recanalization

methods will be engaged for this particular case is made. This requires exchange of the catheter for the particular dedicated device. The interventionalist has to have more endovascular devices in store, since every patient is unique and the recanalization must be well tailored for the particular situation. Usually, 50 I.U. per kilogram (max. 3000 I.U.) of Heparin® are administered at the beginning of the procedure to prevent clot formation on introduced equipment. Furthermore, heparinized saline is used to continually flush all coaxial systems. Although some authors discourage the use of Heparin®, the authors have not encountered any substantial bleeding into the ischemic brain tissue over the past six years.

To conclude, the way of microcatheter introduction into intracranial vessels is standardized at the authors' institution and does not differ much between various intracranial endovascular procedures performed with different devices.

After successful artery reopening, any possible residual restenosis (or residual stenosis) at the original occlusion site should be precisely evaluated and treated if needed. The other very important issue is to evaluate distal arteries patency. Any distal embolization or residual occlusion has to be excluded. If eligible, patients with reocclusion are treated with IA rt-PA administration. Last, but not least, the proximal parts of the brain supplying vessels should be evaluated and treated if needed.

CONCLUSION

Early recanalization is associated with a significantly higher chance of independency after 90 days, with a significant reduction of mortality. In addition to pharmacological methods (IVT and IAT), mechanical neuro-interventional methods are intensively tested and introduced into clinical practice. Results of first studies showed safety and efficacy of various devices with increasing recanalization rate and relative low number of peri-procedural complications and SICH. However, clinical benefit for acute stroke patients has to be confirmed in prospective studies.

ACKNOWLEDGMENT

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Chapter 49

METABOLIC SYNDROME, SUBCLINICAL CAROTID ATHEROSCLEROSIS AND RISK OF STROKE: HOW STRONG IS THE LINK?

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ABSTRACT

The metabolic syndrome (MetS) is a distinctive phenotype with clustering of risk factors associated with an increased risk of vascular disease. MetS is reaching epidemic proportions worldwide. MetS and its components (hypertension, insulin resistance, dyslipidemia, and obesity) are established risk factors for stroke. Surrogate markers of subclinical carotid atherosclerosis such as carotid intima-media thickness (cIMT), carotid plaque (CP) presence and morphology, and carotid artery stiffness (STIFF) also have been linked to an increased risk of stroke. As multiple risk factors increase risk of vascular disease more than the sum of accompanying single risk factors, MetS has significant effects on development of atherosclerosis. Proinflammatory state and oxidative stress induced by MetS play a pivotal role in the carotid artery wall damage. MetS is an independent determinant of cIMT in both sexes, and predicts cIMT progression in large cohorts comprised of different race-ethnicities. Moreover, MetS has been demonstrated to significantly increase STIFF and to be a significant predictor of CP in multi-ethnic populations. Controversially, presence of MetS was not associated with cIMT and CP independent of other cardiovascular risk factors in individuals with optimal, normal, or high-normal blood pressure. Recently, a major issue has been whether MetS is a valid indicator of vascular risk, although from a clinical perspective, it serves a useful purpose to focus on individuals at high risk for developing vascular disease and type 2 diabetes. The development of non-invasive ultrasound methods capable of quantitatively evaluating atherosclerosis has provided an opportunity to

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understand the early determinants of atherosclerosis and has improved our ability to detect individuals at high risk for stroke including individuals with MetS. Therefore, exploring and understanding the link between MetS and subclinical atherosclerosis could help further risk-stratify individuals with MetS, design new strategies to prevent stroke, and to determine the predominant components of MetS associated with carotid atherosclerosis in order to develop new and effective antiatherosclerotic therapies.

1. WHAT IS METABOLIC SYNDROME?

Metabolic syndrome (MetS) is a complex disorder considered a worldwide epidemic. [1] MetS is defined by a cluster of interconnected factors that increase the risk of atherosclerosis, cardiovascular diseases (CVD), stroke, and diabetes mellitus type 2 (DMT2). Its components are dyslipidemia (elevated triglycerides and apolipoprotein B (apoB)-containing lipoproteins, and low high-density lipoproteins (HDL)), elevation of arterial blood pressure (BP) and impaired glucose homeostasis, with abdominal obesity and/or insulin resistance (IR). [2]

More recently, other factors such as proinflammatory state, oxidative stress, and non-alcoholic fatty liver disease have been suggested to play an important role in MetS, making its definition even more complex. [2] Therefore, besides its clinical implications, there is still no a full consensus on pathogenic mechanism or defined diagnostic criteria for MetS. The scientific community still is debating whether MetS represents a specific syndrome or just a surrogate of combined risk factors that increase the risk for stroke, CVD and DMT2.

Since the first definition of Syndrome X given by Reaven that hypothesized a central role of vascular risk factors in the development of CVD and DMT2, [3] many international organizations and expert groups, such as the World Health Organization (WHO), the European Group for the study of Insulin Resistance (EGIR), the National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATPIII), the American Association of Clinical Endocrinology (AACE), the International Diabetes Federation (IDF), and the American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI), have attempted to incorporate the different parameters to define MetS (definitions from these organizations have been well reported on [2]).

Briefly, in 1998 the WHO proposed that MetS may be defined by the presence of insulin resistance (IR) or DMT2, as essential components of the syndrome, along with at least two of the following parameters: raised BP, hypertriglyceridemia and/or low HDL-cholesterol, obesity (as measured by waist/hip ratio (WHR) or body mass index (BMI)), and microalbuminuria. [4] Subsequently, the EGIR excluded microalbuminuria from the syndrome while included hyperinsulinemia, and waist circumference (WC) as the main indicator to assess obesity. [5] In 2001, the NCEP:ATPIII published new criteria that included WC, blood lipids, BP, and fasting blood glucose (FBG).[6]

The IDF along with AHA/NHLBI particular emphasized abdominal obesity as a prerequisite of the diagnosis of MetS [7,8] with the goal to better predict risk for stroke, CVD, and DMT2. However, abdominal obesity was defined differently: the IDF recommended that the threshold for WC in Europeans should be 94 cm for men and 80 cm for women, while the AHA/NHLBI recommended cut-off points of 102 and 88 cm, respectively.

Currently, the two most used definitions for MetS are those of the NCEP:ATPIII and IDF specifically focused on WC as a surrogate measure of central obesity. However, a major

problem with these definitions has been their applicability to different ethnic populations, especially when trying to define obesity cut-off values. For example the risk of DMT2 is different across the Western and Eastern populations. To overcome these difficulties the Joint Interim Statement [9] suggested that there should not be an obligatory component, but that waist measurement would continue to be a useful preliminary screening tool. Three abnormal findings out of the proposed 5 would qualify a person for the MetS.

Based on these findings and considering the high incidence of stroke, CVD and DMT2, more practical and useful definitions for MetS should be proposed. The updated clinical guidelines for cholesterol testing and management (ATPIV) from the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults are under development and due for publication in 2012. http://www.nhlbi.nih.gov/guidelines/cvd_adult/background.htm

2. METABOLIC SYNDROME AND RISK OF STROKE AND DIABETES

Metabolic Syndrome and Risk of Stroke

CVD is an important outcome in subjects with MetS. In fact, all the components of the various MetS definitions are involved in conferring risk of stroke and CVD. As expected, IR is more related to the risk of developing DMT2. In our study from a population-based multi-ethnic cohort of Northern Manhattan (the Northern Manhattan Study-NOMAS) in 1,509 individuals free of stroke, myocardial infarction (MI), and diabetes, IR, estimated by homeostasis model assessment (HOMA), was associated with a 2.8-fold increased risk of first ischemic stroke. [10] Using the same study population we have also showed the association between FBG and risk of stroke [11] and demonstrated that FBG was an independent predictor of vascular outcomes among individuals without diabetes. [12] Dyslipidemia and hypertension have been more associated with risk of stroke. [13,14] WC has been demonstrated to be associated with both stroke and DMT2. [15]

Several epidemiological studies have confirmed the increased risk of stroke in individuals with MetS, regardless of the diagnostic criteria used. The adjusted risk ratios for incident ischemic stroke associated with MetS range between 2.1 and 2.5 in prospective studies, and a hazard ratio as high as 5.2 has been reported. [16-18] Koren-Moraq et al. [16] using a cohort of 14,284 patients, demonstrated that patients with MetS without diabetes exhibited a 1.49-fold increased risk of ischemic stroke or transient ischemic attack (TIA), whereas those with frank diabetes had a 2.29-fold increased risk. The relative odds for ischemic stroke or TIA, associated with presence of MetS, were 1.39 in men and 2.10 in women. In the prospective Framingham cohort, both diabetes and the MetS were powerful risk factors, with a 10-year risk of ischemic stroke associated with diabetes of 14% in men and of 10% in women compared with 8% and 6% in nondiabetic men and women, respectively, with the MetS alone. [17] In a study where MetS was defined by the ATPIII criteria in 1,131 subjects with no history of CVD and diabetes, those with MetS had a 2.05-fold risk for all stroke and 2.41-fold risk for ischemic stroke, after adjusting for socioeconomic status, smoking, alcohol, and family history of coronary heart disease (CHD). The risk ratios among patients with MetS as defined by the WHO criteria were 2.08 and 2.47. [18] Although the data are similar across

studies, some still argue that the results from studies using different MetS definitions are not exactly comparable.

In NOMAS we found a significant association between the MetS and ischemic stroke risk, independent of other confounding factors including age, education, physical activity, alcohol use, and current smoking. [19] The prevalence of MetS in NOMAS was 49%, and differed by sex (39% in men, 55% in women, $p < 0.0001$) as well as race-ethnicity (56% in Hispanics, 41% in blacks, and 39% in whites, $p < 0.0001$). Interesting, the effect of the MetS on stroke risk was greater among women (HR=2.0; 95% CI, 1.3 to 3.1) than men (HR=1.1; 95% CI, 0.6 to 1.9) and among Hispanics (HR=2.0; 95% CI, 1.2 to 3.4) compared to blacks and whites. The etiologic fraction analysis estimated that elimination of MetS would result in a 19% reduction in overall stroke, a 30% reduction of stroke in women and a 35% reduction of stroke among Hispanics. Subsequently, using the same study population we confirmed that MetS was independently associated with all cardiovascular events, especially in subjects with both MetS and endothelial dysfunction.

Overall, these data strongly suggest that the risk of any incident stroke is increased in people with MetS and without history CVD and diabetes. [18]

However, fewer studies reported on the comparisons between MetS, its components, and various risk prediction scores on the risk of stroke and CVD. [20,21] In the Atherosclerosis Risk in Communities (ARIC) study, men and women with MetS were approximately 1.5 and 2 times more likely to develop ischemic stroke than control subjects after adjustment for age, smoking, LDL cholesterol, and race-ethnicity. However, comparison of receiver operating characteristic curves indicated that MetS did not considerably improve vascular risk prediction beyond the level achieved by the Framingham Risk Score (FRS). [21]

The reasons for discrepancy in these results are not quite clear but most likely they may have been due to the different cohorts studied, differences in the study designs (prospective vs. case control), as well as different MetS criteria used. To further explore these controversies, a meta-analysis was conducted on 21 prospective studies and showed that MetS was associated with the increased mortality from all CVD causes as well as with an increased incident CVD (RR 1.53; 95% CI, 1.26-1.87), CHD (RR 1.52; 95% CI, 1.37-1.69) and stroke (RR 1.76; 95% CI, 1.37-2.25). [22] The relative risk of CVD associated with the MetS was higher in women compared with men.

Putting together these findings, it is evident that patients with MetS have a greater risk of incident of stroke and other vascular diseases. Therefore we agree and support the proposed statement that *“a major breakthrough related to the concept of the MetS is the recognition of the high cardiovascular risk in subjects with a cluster of mild abnormalities or with a cluster of abnormalities that are not regarded as driving forces in CVD”*. [23]

Metabolic Syndrome and Risk of Diabetes

Patients with diabetes are at significant risk for CVD and vascular death. Approximately 65% of deaths in patients with diabetes are attributable to heart attack or stroke. [24] It is now well documented that MetS not only increases the risk for DMT2, but is also highly predictive of new onset DMT2. [25] Since impaired FBG and impaired glucose tolerance are components of the ATPIII and the WHO definitions of MetS respectively, this finding is not surprising. However some controversy still exist. A study conducted in 1,448 nondiabetic

individuals, demonstrated that the three components of the MetS were significant associated with risk of DMT2. These were insulinemia, the body size and lipids, whereas the BP was not associated with risk of DMT2. [26]

MetS criteria modify the ability of MetS in predicting DMT2. In 1,734 participants from the San Antonio Heart Study, [27] MetS predicted DMT2 independently of other factors. The ATPIII definition of MetS may perform better than the modified WHO definition. In a meta-analysis [28] the random-effects summary were 5.17 for the WHO definition (10 cohorts); 4.45 for the EGIR definition (4 cohorts); 3.53 for the ATPIII definition (13 cohorts); 5.12 for the AHA/NHLBI definition (5 cohorts); and 4.42 for the IDF definition (9 cohorts). These data suggest that a greater number of abnormal MetS components was strongly related to incident diabetes.

The major limitations to the results of these studies are the lack of accounting for other important factors that may be implicated in developing of diabetes. In particular, proinflammatory state and oxidative stress have been demonstrated to play a pivotal role in DMT2. [29,30] It is probable that along with the components of MetS described below, other mechanistic pathways may account for the residual CVD risk in MetS.

3. METABOLIC SYNDROME AND RACE-ETHNICITY

The prevalence of the MetS varies among ethnic groups. [31-33] According to the Third National Health and Nutrition Examination Survey, adult prevalence of the MetS was 32% in Hispanic-Americans, 22% in African-Americans and 24% in Caucasian-Americans. [32] Similarly, a prevalence of 24% among white subjects was reported in the Framingham Offspring Study, and a prevalence of 31% was reported among Mexican-American subjects in the San Antonio Heart Study. [33] Similar percentages have been reported also in other populations. [34,35]

Based on these findings it seems that blacks have a lower prevalence of MetS compared with other race-ethnicity. This is surprising since each of the MetS components is more frequent in African-Americans than in Caucasians. Several have been the proposed explanation for this paradox. It may be partly ascribed to the differences in IR and serum triglycerides in blacks when compared with other race-ethnicities. [36] In contrast, Hispanics have a high prevalence of the MetS compared to white and blacks. [37] One reason proposed is the high rates of IR, DMT2, and the prevalence of abdominal obesity and high triglycerides among Hispanics. [37]

Similar difficulties in using the MetS criteria were present also in analyses of Eastern populations. For instance, Asians tend to develop DMT2 while still maintaining “normal” WC values. [38] Therefore, applying the ATPIII guidelines to Asians was criticized for inaccurate estimation the prevalence of MetS.

However, the real reasons for the racial/ethnic differences in MetS prevalence still remain unknown. It is possible that the current definitions, criteria, and cut-off points underestimate the burden of components of MetS in different race-ethnicities. Therefore, it is important to reconsider the current classification of MetS by the NCEP, WHO, and IDF based on the characteristic of the studied populations. [39] In addition, besides variations in environmental factors, a different genetic susceptibility could explain these observed differences.

In a cohort consisted of 803 subjects from 89 Caribbean-Hispanic families enrolled in the Family Study of Carotid Atherosclerosis from NOMAS, we found a significant heritability for MetS and each of its components. [40] After adjusting for age and sex, the MetS had a heritability of 24% ($p=0.009$). When the components were treated as continuous traits, the estimates of heritability varied from 16% for systolic BP to 60% for HDL-cholesterol after adjusting for age, sex and appropriate medications. When the MetS components were analyzed as discrete traits according to ATPIII, the estimates of age and sex-adjusted heritability varied from 27% for large WC to 51% for low HDL-cholesterol levels. Other studies reported similar heritability estimates. [41,42] This evidence confirm that genetic factors contribute to the familial aggregation of the MetS and its components, and this may in part explain the variability of MetS incidence and prevalence in different race-ethnicity populations.

4. SUBCLINICAL CAROTID ATHEROSCLEROSIS AND RISK OF STROKE AND CARDIOVASCULAR DISEASE

Atherosclerosis is a physical manifestation of many of risk factors associated with stroke, such as hypertension, dyslipidemia, diabetes mellitus, obesity, that are all components of MetS. Carotid atherosclerosis accounts for a large percentage of both embolic and ischemic strokes. The development of noninvasive ultrasound methods capable of quantitatively evaluating atherosclerosis has improved our ability to design studies analyzing the natural history, progression, and determinants of carotid atherosclerosis, as well as to evaluate the effects of preventive measures. The most validated carotid traits currently used to evaluate CVD risk prediction are: carotid intima media thickness (cIMT), carotid plaque (CP) and its phenotypes, and carotid stiffness (STIFF).

Carotid Intima Media Thickness (cIMT) and Risk of Stroke and CVD

cIMT may be defined as a measurement of the thickness of the carotid artery walls. cIMT is increasingly used as a surrogate end point of vascular outcomes in clinical trials aimed at determining the success of interventions that lower risk factors for atherosclerosis and associated diseases such as stroke. [43] In a systematic meta-analysis, Lorenz et al. reviewed 8 observational studies conducted on a total of 37,197 subjects with carotid ultrasound data who were followed up for a mean of 5.5 years. For an absolute cIMT difference of 0.1 mm, the future risk of myocardial infarction (MI) increased by 10% to 15%, and the stroke risk increases by 13% to 18%. The risk for both end points decreased with the number of adjustments for risk factors. [44] Another study reviewed prospective epidemiological data in the general population, to determine the association of cIMT with cardiovascular risk. The main conclusions were that cIMT was an independent but relatively modest predictor of CHD, instead cIMT was an independent predictor for stroke, slightly better than for CHD as judged by the relative risks of both events. [45]

Despite these evidences, recently the enthusiasm has been decreased by the result of a recent meta-analysis conducted in 41 trials enrolling 18,307 participants. This study

demonstrated as the regression or slowed progression of cIMT, induced by cardiovascular drug therapies, do not reflect reduction in cardiovascular and cerebrovascular events. [46] In addition, variability in predicting risk of stroke varied across the cIMT measured in different carotid segments. For example, in 1,975 Framingham Offspring Study participants, investigators demonstrated that internal cIMT, but not common cIMT, was associated with higher prevalence of cerebral infarcts. [47] However, a study conducted in 5,056 patients enrolled in the Carotid Atherosclerosis Progression Study (CAPS) demonstrated that cIMT measured in common carotid artery, bifurcation, and internal carotid artery was highly predictive for stroke even after adjustment for age, sex, and vascular risk factors. Its predictive value was at least as high in younger subjects as in older subjects. [48] Interesting, recent findings from the PROG-IMT suggested that estimation of individual IMT progression may be more useful in predict CVD than the singular, one time point, IMT measurement. [49]

It is important to note that pathological changes of arterial medial hypertrophy or intimal thickening in the absence of atherosclerosis are not a real representation of atherosclerosis. However, because cIMT and atherosclerosis share common underlying mechanisms for both disease initiation and progression, cIMT may be better indicator or overall risk of stroke and CVD than an accurate measure of atherosclerosis per se. [50]

Carotid Plaque (CP) and Risk of Stroke and CVD

CP is a focal manifestation of atherosclerosis and is biologically and genetically different phenotype of atherosclerosis from cIMT. [51,52] Therefore, it is considered as an independent surrogate marker of vascular disease. Atherosclerotic plaques that are prone to rupture owing to their intrinsic composition such as a large lipid core, thin fibrous cap and intraplaque hemorrhage are associated with subsequent thromboembolic ischemic events. [53] Similar to cIMT, presence of stenotic atherosclerotic CP is a well-established risk factor for IS and CVD. [54,55]

In NOMAS, subclinical CP was a strong predictor of vascular outcomes. [56] In this study, we described the impact of CP stratified by traditional FRS categories on vascular outcomes, showing that about 30% of subjects in low and 42% in intermediate FRS category had CP. In addition, we found that CP with irregular surface increased the risk of ischemic stroke 3-fold. [57] The cumulative 5-year IS risk among individuals with an irregular plaque surface was over 8%, whereas those with regular plaque had <3%. These data suggested that plaque surface irregularity, even after adjusting for degree of stenosis and plaque thickness, is an independent predictor of stroke. These data from NOMAS were in agreement with the Rotterdam Study [58] where in an old population, presence of CP increased the risk of stroke and cerebral infarction about 1.5-fold, irrespective of plaque location.

Other studies conducted on NOMAS population showed that the calcified CP was an independent predictor of combined vascular events defined as ischemic stroke, MI or vascular death. [59] Moreover, we demonstrated that people with maximum carotid plaque thickness (MCPT) greater than 1.9 mm had a 2.8-fold increased risk of combined vascular events in comparison to the subjects without CP. Interesting, in this study, the amount of atherosclerosis overall was higher among whites and blacks than among Hispanics, but if CP was present, it had a significant impact on the vascular risk only among Hispanics.

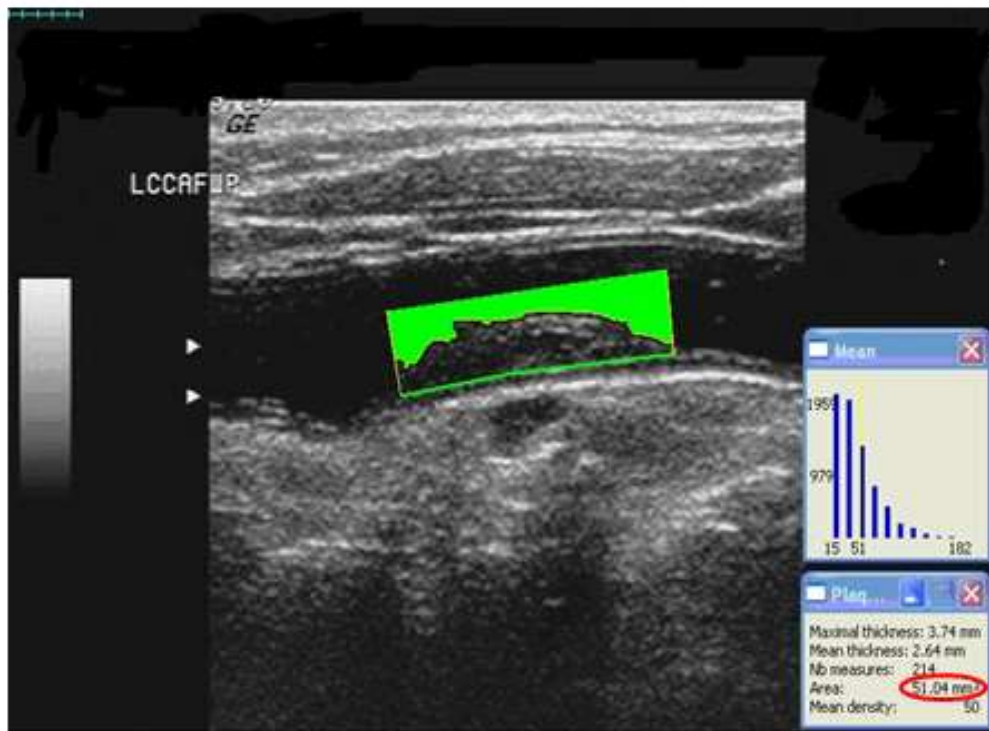


Figure 1. B-Mode ultrasound measurement of plaque area in the carotid artery. Plaque area is measured by automated edge detection algorithm in defined segment of the carotid artery (the green box) with visualized atherosclerotic plaque (coded gray in the green box). Measured plaque area is 51.04 mm² (circulated in red in the view box located in the bottom right corner).

In fact, in fully adjusted models, the association between CP and vascular events was significant only among Hispanics, suggesting a role of race-ethnicity in the CP related risk of CVD.

Recently, some plaque's echographic parameters have shown to help detecting increased vascular risk. Currently, new evidence suggests that total plaque area is the strongest predictor of cardiovascular risk among the ultrasound phenotypes (plaque presence, thickness or cIMT). [60]

Taking together, these data support strong association between CP, stroke and CVD. However, the comparison of the results from various populations must be considered with caution. Differences in demographics and risk factor profiles across different study populations and use of different measurements of subclinical atherosclerosis may have contributed to varying plaque size and its associated risk of vascular events.

Arterial Stiffness (STIFF) and Risk of Stroke and CVD

STIFF is a measurement of the vessel wall's tendency to resist deformation by systolic BP during the cardiac cycle, and is considered a parameter of the arterial distensibility. [61] STIFF is greater among people with atherosclerosis, and is considered an early predictor of stroke and CVD. [62]

Functional properties of the arterial wall precede the clinical stage of atherosclerosis has been investigated in peripheral arteries (femoral and brachial) and in the aorta for many years by using different methodologies such as measurement of pulse wave velocity (PWV). Recent development of high-resolution and high-definition sonography has focused new investigations on the carotid arteries.

In a cohort of 79 end stage renal disease (ESRD) patients with a mean follow-up of 25 months, increased STIFF was associated with a 6.4-fold increased risk of CVD and all-cause mortality, independent of other prognostic factors such as diastolic BP and total cholesterol/HDL ratio. [63]

In a case-control study conducted in 267 stroke patients, carotid distensibility (inverse of STIFF) was significantly lower in stroke patients (0.353 mm, 95% CI: 0.326–0.379) than in control subjects (0.415 mm, 95% CI: 0.378–0.451) even after adjusting for BP values, diastolic carotid-diameter and height. Each 1 SD decreased in the carotid distensibility independently increased the likelihood of stroke by 167.0%. [64] Data from the SMART Study (Second Manifestations of ARterial disease) confirmed these findings. [65] In 420 participants with stenosis of > 50% in at least one of the internal carotid arteries, risk of ischemic stroke or TIA in the highest quartile (stiffest arteries) relative to the lowest quartile was 2.1 (95% CI, 1.1 to 4.1) after adjustment for age, sex, systolic BP, minimal diameter of the carotid artery, and degree of carotid artery stenosis. [66]

Several mechanisms may explain the association between increased STIFF and stroke. First, STIFF may favor the occurrence of cerebrovascular disease through an increase in central pulse pressure that influences arterial remodelling at the sites of both the extracranial and intracranial arteries, increasing the development of carotid artery stenosis, the likelihood of plaque ulceration and rupture as well as the incidence of cerebrovascular disease. Furthermore, STIFF may be associated with cerebral microvascular processes important in stroke pathogenesis. [62] In contrast, the Rotterdam Study [67] reported no association between carotid distensibility and CVD. Different populations, methods and analyses may explain the lack of replication of the study results. Nonetheless, as well as with the other carotid markers of atherosclerosis, the genetic component may play a pivotal role in STIFF. In addition, few studies investigated STIFF in different race-ethnicity populations. Recently, we reported race-ethnicity differences in STIFF and arterial diameter in NOMAS population. [68] We found that age was independently associated with greater carotid artery diameter among Hispanics but not among blacks or whites. Interesting, the association between age and STIFF was strongest among Hispanics, followed by blacks, and was not apparent among whites. These findings may offer a possible explanation for reported disparities in stroke morbidity and mortality among Hispanics compared to blacks and whites.

5. THE LINK BETWEEN COMPONENTS OF METABOLIC SYNDROME AND SUBCLINICAL CAROTID ATHEROSCLEROSIS

Each component of MetS has been significantly associated with earliest stages of atherosclerosis. However, in a recent study we demonstrated that traditional vascular risk factors explain only 21% of the variance in the total carotid plaque burden. [69] Therefore variation in subclinical carotid atherosclerosis is largely unexplained by known vascular risk

factors, suggesting that other unaccounted factors, both environment and genetic, play an important role in the determination of subclinical atherosclerotic.

Hypertension and Subclinical Carotid Atherosclerosis

Hypertension through many mechanisms such as impairment of the endothelium and inflammation increases risk of atherosclerosis and stroke. High BP levels along with age are the two major clinical determinants of cIMT and STIFF. In the Framingham Study, systolic BP was independently associated with carotid atherosclerosis in multivariate analysis. [70] The odds ratio for carotid stenosis for an increase of 10 mmHg in systolic BP was 1.22 (95% CI 1.10 to 1.37) in men and 1.21 (95% CI 1.12 to 1.31) in women. Hypertension had a significant effect on different phenotypes of atherosclerosis. A study conducted in 1,031 middle-aged (aged 40-60 years) demonstrated a significant difference in cIMT between the hypertensive and control subjects. Moreover, CP were found more frequently in the hypertensive patients than they were in their controls. Subjects with a long duration of hypertension (> 7 years) had higher prevalence of CP and greater cIMT than those with a short duration of hypertension. [71]

A different impact of hypertension on subclinical carotid atherosclerosis has been demonstrated among different race-ethnicities. In the ARIC among 15,587 middle-aged participants [72] higher hypertension categories were associated with cIMT in all ethnic and sex groups, in participants who did and did not use BP lowering medications. In participants who did not use medications, multiple linear regression models adjusted for diastolic BP and other risk factors found that systolic BP was positively associated with cIMT except in black men; diastolic BP was not related to cIMT in blacks and had a negative relation with cIMT in white men and a J-shaped relation with cIMT in white women.

Increased BP has been associated with STIFF in several studies. Recently, a link between hypertension, endothelial dysfunction and STIFF has been clearly demonstrated in hypertensive patients. [73]

However, only few studies have evaluated the association of hypertension with the occurrence of CP [71,74,75], and the mechanism linking hypertension with plaques remains poorly understood. An interesting study conducted in 1,008 Finns subjects [76] showed close relationship between hypertension, especially high systolic BP, and CP in carotid and femoral arteries. Of the subjects, 64% had CP in both carotid and femoral arteries. The subjects with high (160 mmHg) systolic BP had a three-fold risk for CP in both carotid and femoral arteries compared to those with lower systolic BP. In addition, male sex and long-lasting smoking were risk factors for the occurrence of carotid and femoral plaques.

Based on these evidences, several studies and ongoing clinical trials are currently aimed to evaluate the role of anti-hypertensive drugs in reducing carotid atherosclerosis.

Glucose Homeostasis and Subclinical Carotid Atherosclerosis

IR is a metabolic disorder characterized by diminished tissue sensitivity to insulin that originates from environmental factors, such as a sedentary lifestyle, central obesity, and genetic predisposition. [77] IR was found to accelerate atherosclerosis, inflammation, the

onset of DMT2, CVD, obesity, hypertension, chronic kidney disease, and dyslipidemia. [78] Although an association between DMT2 and subclinical atherosclerosis is evident, [79] however it is still unclear whether IR or FBG are independently associated with subclinical atherosclerosis among non-diabetic individuals. In 3,704 patients with DMT2, insulin sensitivity index but not hyperinsulinemia, was significantly associated with cIMT adjusting for age, sex, BMI, smoking, systolic BP, HDL and LDL cholesterol. [80] These data suggest that IR itself, but not hyperinsulinemia might be an underlying mechanism of subclinical atherosclerosis in patients with DMT2. The HOMA index of IR was also positively associated with CP in subjects without diabetes and CVD, with an odds ratio of 1.19. [81] Other studies confirmed these results demonstrating that both impaired FBG and impaired glucose tolerance patients have increased cIMT according to controls. [82]

The ARIC study reported differences on glucose homeostasis in predicting subclinical carotid atherosclerosis among different race-ethnicities. In overall population STIFF were higher with increasing concentrations of FBG. [83] This finding was consistent in both black and white examines and in both sexes. A 25% increase in FBG was associated in nondiabetic white men with a 5.8% increases in STIFF. In nondiabetic white women, the corresponding predicted changes were an increase of 16.6% in STIFF.

Insulin and higher blood glucose level may have a direct effect on the thickness and structure of the arterial wall. [24] *In vitro* studies have shown that insulin, in concentrations similar to those found in physiologic conditions, stimulates proliferation of cultured arterial smooth muscle cells from a number of species, including humans. [84] Endothelial cells cultured from large vessels are resistant to the actions of insulin, but hyperglycemia inhibits their proliferation. [84] These findings suggest that hyperglycemia and hyperinsulinemia may promote the development of atherosclerosis. However, the exact mechanisms are not fully understood.

Dyslipidemia and Subclinical Carotid Atherosclerosis

Dyslipidemia is considered the most prevalent and modifiable risk factor for atherosclerosis. Abnormalities of lipid metabolism lead to pathologic lipid accumulation in the vessel wall, proinflammatory effects in macrophages and chronic oxidative stress of endothelial cells, ultimately leading to the formation of atherosclerotic lesions. [85] As component of MetS is defined as: elevated triglycerides and apolipoprotein B (apoB)-containing lipoproteins, and low HDL. Serum lipoprotein (Lp) levels are significantly higher in patients with CP or cIMT than in controls without. [86] However, no significant relationship between Lp level and arterial endothelial function, smooth muscle response, or carotid wall thickness, even though other lipid risk factors like LDL and HDL ratio are correlated with abnormal arterial function and structure. [86]

The anti-atherogenic property of HDL has been documented in both clinical observations and laboratory settings. [87] Also the association between the ApoB and development of atherosclerosis evaluated as cIMT has been shown. [88] The Bogalusa Heart Study in a young cohort of 1,203 black and white subjects demonstrated that only non-HDL cholesterol, total cholesterol/HDL cholesterol, and ApoB emerged as significant correlates with cIMT, after adjusting for BMI, systolic BP, and other lipoprotein. [89]

Due to the complexity of lipid effects on atherosclerosis, we hypothesize that the ratio of lipid parameters, rather than single lipid parameters, was most associated with CP phenotypes. [90] In NOMAS, our results indicate a strong relation between blood lipid levels and presence of CP in a multi-ethnic population, suggesting that blood lipids likely influence the risk of CVD and particularly stroke due to their effects on atherosclerotic plaque formation. Elevated LDL was independently associated with CP. However, HDL and triglycerides were not significantly associated with plaque presence after controlling for other lipid parameters and vascular risk factors. An analysis of precursor proteins supported the strength of the LDL association. ApoB as the LDL precursor protein, was associated with an increased risk of plaque, whereas ApoA-I, the HDL precursor protein, was not. In fact, the lipid parameter most predictive of plaque presence was ApoB, and particularly the ratio ApoB:ApoA-I.

Other studies have also demonstrated no significant association between triglycerides and CP after controlling for other lipid parameters and vascular risk factors. [91,92]

Studies examining the effects of lipid-lowering treatments have provided strong evidence for an important role of lipids in the pathogenesis of carotid atherosclerosis. A meta-analysis suggested that the effect of statins on both risk of stroke and cIMT progression was closely associated with the reduction in LDL cholesterol. [93] Other recent studies demonstrated that short time treatment with lipid-lowering drugs may modify the CP morphology, [94] and reduce cIMT progression. [95]

Obesity and Subclinical Carotid Atherosclerosis

The association between obesity and CAD is well established. Obesity represents a chronic inflammatory status and adipocytes release either cytokines or an array of adipokines such as leptin, endowed with immunomodulating and systemic activities leading to atherosclerosis. Recently, the new concept of “obesity paradox” has been introduced. [96] Data from the PROFESS (Prevention Regimen for Effectively Avoiding Second Strokes Trial) [97] that reviewed 20,246 subjects stratified by their BMI and WC, found no statistically significant differences in the recurrence of stroke for lean (9.06%), overweight (8.82%), and obese (8.89%) groups over 2.5-year follow-up. In contrast, in NOMAS we stressed the importance to include WC to the traditional Framingham profile to better improve global vascular risk prediction. [98]

The role of obesity measured by BMI and WC in subclinical atherosclerosis is also controversial. The Cardiovascular Disease Prevention Campaign analyzed 840 female and 1,002 male participants divided into normal weight (BMI <25 kg/m²), overweight (BMI between 25 and 29.9 kg/m²) and obese (BMI >29.9 kg/m²). The results suggested that increasing body weight did not independently associate with carotid atherosclerosis. [99] Another study compared carotid atherosclerosis prevalence measured as cIMT in obese postmenopausal women with and without MetS. The results found that women with MetS had higher cIMT prevalence in respect to women without MetS. However, obesity per se was not associated to carotid atherosclerosis. [100] One of the reasons for a lack of the association may be that BMI may not truly reflect body fat distribution. WC, WHR, and skin-fold thickness have been shown to provide better information in addition to BMI. WC is considered a risk marker that reflects long-term deviations in several metabolic risk variables.

Visceral fat is more metabolically active and associated with inflammation, IR, and atherosclerosis. [101] WC was independently associated with CP and progress of cIMT in peritoneal dialysis patients. [102] In an observational study performed in 3,694 Korean patients with DMT2, cIMT and the frequency of CP were higher with increasing WC quartiles. [103] In contrast, a recent study demonstrated no statistical differences in STIFF between groups divided for WC values. [104]

Environmental and genetic factors may play an important role in the relationship between obesity and CVD. We evaluated the genetic and environmental contributions to cIMT and obesity in 440 subjects from 77 Caribbean Hispanic families. [105] Obesity phenotypes included BMI, WC, WHR, and skin-fold thickness. We found a substantial genetic contribution to cIMT, BMI, WC, and skin-fold thickness. Significant phenotypic correlations were found between cIMT at each segment and the 3 obesity phenotypes (BMI, WC, and skin-fold thickness). These results suggest that obesity and cIMT may share common genetic factors.

A debate regarding the obesity paradox and which measurement of obesity is more helpful in predicting atherosclerosis, stroke and CVD is still ongoing.

6. LINK BETWEEN METABOLIC SYNDROME AND SUBCLINICAL CAROTID ATHEROSCLEROSIS

A lively debate is currently ongoing as to whether MetS per se could impact carotid artery atherosclerosis. As we reviewed in the previous sections, all features of MetS are important risk factors for atherosclerosis, stroke and CVD. Several studies investigated the possible association between MetS and subclinical carotid atherosclerosis. In the ARIC [106], a large community-based cohort (more than 14,000 middle-aged adults), individuals who met the criteria for MetS had cIMT values 30 μm (95% CI, 10–50 μm) thicker than normal individuals. Subsequently, the Bruneck study [107] conducted in a cohort of 888 Italian subjects reported a significant association between MetS and CP progression, assessed as a change in CP score over 5 years of follow-up. In the same study, the plaque progression was also associated with increased risk of CVD. The Baltimore Longitudinal Study of Aging (BLSA) examined the relationship between MetS and cIMT and STIFF. [108] In 471 subjects free of clinical overt CVD at baseline, investigators found that MetS increases cIMT and STIFF across all age groups; and MetS exerts its effects on carotid structure and function independent of its individual components and other CVD risk factors. These findings were in agreement with the study in patients with the IR syndrome. [109] Recently in a cohort of 400 Chinese subjects, [110] MetS significantly increased cIMT and significantly impaired carotid elastic properties. Interesting, among the MetS components WC had a strong association with carotid atherosclerosis.

In NOMAS, we evaluated the relationship between MetS and subclinical atherosclerosis measured as CP presence. [111] We observed an independent association between the MetS and CP prevalence and MCPT. Further, CP prevalence and MCPT were significantly related to the number of MetS components found within each individual. Our study was also the first to show that MetS was related to carotid atherosclerosis in whites, blacks, Hispanics, men, and women in a single community-based elderly cohort. The relationship between MetS and

CP was somewhat stronger in men than in women, was weaker in white women, and particularly stronger in white and black men. The genetic mechanism may explain at least in part these results, especially among postmenopausal women in whom the atheroprotection of sex hormones is lost.

In NOMAS, we also demonstrated a significant association between MetS and STIFF. [112] This relationship was independent of sex and race-ethnicity. In addition, it was independent of presence of CP and cIMT, and lipid lowering and BP lowering agents that could affected artery wall function. Wider WC and elevated BP were MetS components most significantly associated with STIFF.

Despite these evidences, the debate regarding the role of MetS in subclinical carotid atherosclerosis continues. Recently, the Cardiovascular Risk in Young Finns Study [113] showed that despite all MetS definitions identified young adults with accelerated cIMT progression, MetS was not able to predict cIMT progression more than expected from the sum of its risk components.

Interesting sex-specific differences were reported for the relationship between MetS and carotid atherosclerosis. [114,115] In The Health 2000 Survey [115] MetS was an independent determinant of cIMT in both sexes. However, in women but not in men, MetS was associated with cIMT independently of its components suggesting that MetS provides additional information on a person's risk for early atherosclerosis beyond Framingham Risk Score in women but not in men. Other studies conducted in different race-ethnicity populations supported the associations between MetS and carotid atherosclerosis. [116,117]

Several mechanisms have been proposed for the link between MetS and subclinical carotid atherosclerosis. One well documented deleterious effect is the adverse role on the structural and functional properties of the vasculature such as thickness and stiffness. It is possible that a common pathogenic factor could underlie both the arterial structural changes and the alterations in the components that comprise MetS. In addition, glycation of matrix proteins, nitric oxide regulation and other molecular pathways have been implicated in endothelial damage mediated by MetS. [110]

Incidence of MetS is higher in the older population. [118] The age-adjusted prevalence of the MetS is estimated at 23.7% of the US population and increases to 43.5% of adults >60 years of age. Ageing renders the endothelium more susceptible to damage by insults. [119] Furthermore, viscoelastic properties, such as arterial wall thickening, increased elasticity, reduced compliance and increased STIFF, deteriorate with aging. [120] Therefore, when present, age is another important component in addition to the classical MetS components mediating the arterial damages and the risk for stroke and CVD. However, the exact underlying mechanism remains obscure.

Recently, other factors such as inflammation and oxidative stress have been involved in development of MetS. [110] Oxidative stress develops in hypertrophied adipocytes, which increase the synthesis of pro-inflammatory cytokines, while decreasing anti-inflammatory cytokines. Dysregulation of such adipocytokines is responsible for systemic inflammation and oxidative stress and contributes to the pathogenesis of the obesity-associated morbidity in MetS. [121] All of these mechanisms are linked with atherosclerosis and risk for CVD.

Further experimental and clinical studies are imperative to elucidate the real impact of MetS and its components on the subclinical carotid atherosclerosis.

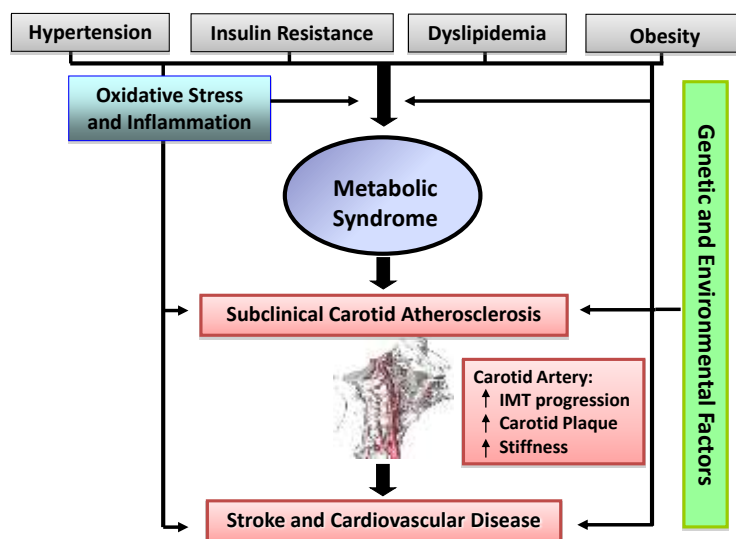


Figure 2. Schematic diagram of the association between the MetS and its components, subclinical carotid atherosclerosis, stroke and CVD, and the interaction with oxidative stress, inflammation and genetic and environmental factors.

7. SUBCLINICAL CAROTID ATHEROSCLEROSIS AS POTENTIAL MARKER TO RISK-STRATIFY PEOPLE WITH METABOLIC SYNDROME

Because of the increased morbidity and mortality associated with MetS, along with the significant direct and indirect economic costs of the associated diseases, the health care system worldwide should urgently seek to initiate programs that identify subclinical disease and create aggressive treatment plans based on risk stratification. A major challenge associated with primary prevention of stroke just involves the early and accurate detection of subclinical carotid atherosclerosis in a population that is at risk but asymptomatic. Delay in identifying at risk populations may result in a missed opportunity to prevent a cardiovascular event, such as heart attack, stroke, or sudden death. Therefore, early detection of subclinical carotid atherosclerosis could help to prevent these vascular events and substantially reduce the levels of death and disability attributable to stroke. This goal may be furthered by developing, standardizing, validating, and disseminating noninvasive diagnostic techniques such as ultrasound methods for identification of stratified risk factors in the general population.

It has been reported that when subclinical disease develops, the traditional risk factors appear to have a smaller association with the subsequent development of clinical disease,

although the traditional risk factors are the primary determinants of subclinical disease. [122] Individuals with the MetS had a higher burden of subclinical carotid atherosclerosis, suggesting that markers of subclinical atherosclerosis are intermediate conditions before occurrence of stroke. From the data presented in the previous sections we may state that: 1. there is not a full consensus of MetS definition; 2. MetS is a risk factor for stroke and diabetes in variable manner, also depending on race-ethnicity; 3. each component of MetS is an important risk factors for stroke; 4. cIMT, CP, and STIFF are well validated markers of subclinical carotid atherosclerosis and prognostic factors for stroke and CVD; and 5. there is a strong link between subclinical carotid atherosclerosis traits and MetS, but variable link with each MetS component.

The fact that more components of MetS an individual possess, the greater is the risk of carotid atherosclerosis, suggests that the clustering of these components is important and that measuring carotid atherosclerotic burden may be a good indicator of risk of stroke. However, the variability of the criteria used to define MetS, and the different characteristics of the study populations make difficult evaluating the risk for stroke in patients with MetS generating a “detection gap”. The current “detection gap” between MetS patients at risk of stroke could be addressed using a combination of noninvasive surrogate markers of atherosclerosis, such as cIMT, CP, and STIFF, alongside traditional risk assessment (ie, Framingham scores).

In addition, repeated noninvasive measurement of surrogate markers may be useful in monitoring therapy in patients with a complex syndrome such as MetS. Prospective studies are need to establish the extent to which measurement of surrogate markers would add value when used in tandem with the traditional risk modification. Such studies should also help establish goals for initiating and monitoring therapies for stroke risk modification.

Moreover, the use of noninvasive surrogate markers in clinical trials may reduce the duration of the clinical trials, as well as the costs associated with conducting these trials. Just few clinical trials aimed to investigate the relationship between MetS and atherosclerosis have been performed and are ongoing. As a part of Hämeenlinna Metabolic Syndrome Research Program (HMS) (NCT01119404) surrogate indicators for atherosclerosis (biochemical markers for endothelial damage) are studied in 120 men with MetS, 120 men with CVD and 80 physically active controls and in different settings. A purpose of an ongoing clinical trial (Early Vascular Wall Changes by Magnetic Resonance Imaging (MRI) in Metabolic Syndrome Versus Metabolically Normal Pre-Menopausal Women: A Pilot Study; NCT01068535) is to identify novel markers of early atherosclerosis in young women by comparing the vascular permeability of blood vessel walls (of the carotid artery) in pre-menopausal women who have MetS versus healthy women.

In conclusion, patients with MetS have a disproportionately high risk of stroke morbidity and mortality. Individuals with MetS should be evaluated for subclinical cardiovascular disease, which is preferably detected by non-invasive ultrasound imaging markers of subclinical atherosclerosis. There is a strong evidence that MetS is linked to increased levels of vascular damage and early detection of vascular changes leading to overt vascular disease is an important step in early and effective prevention of stroke and CVD disease.

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Chapter 50

CONJUGATED LINOLEIC ACIDS IN THE PREVENTION OF ISCHEMIC STROKE

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ABSTRACT

Epidemiological and case-control studies show that high dietary intake of conjugated linoleic acids (CLA) is associated with a reduced risk of ischemic stroke. Atherosclerosis, a major risk factor for ischemic stroke, is precipitated by immune processes which involve the interplay between monocytes/ macrophages, pro-inflammatory and chemotactic mediators at the site of vascular injury. Sparse clinical evidence showed that CLA supplementation had no effect on a surrogate measure of atherosclerosis. However, animal models show significant CLA isomer-specific regression and resolution in established atherosclerosis. Contrary to expectations, clinical trials found that linoleic acid supplementation actually increased plasma levels of pro-inflammatory markers, such as C-reactive protein, IL-6 and TNF- α . In contrast, *in vitro* studies show that linoleic acids inhibited the expression of pro-inflammatory mediators by modulating intracellular signaling pathways. The results of *in vitro* studies are inconclusive regarding the effect of linoleic acids on chemotactic mediators which are critical in the initiation and progression of atherosclerosis. In conclusion, observational evidence points to an inverse relationship between dietary CLA intake and the risk of stroke. However, the findings from clinical trials and laboratory studies are inconclusive and do not provide a clear understanding of the mechanisms underlying protection against ischemic stroke.

LIST OF ABBREVIATIONS

CLA	Conjugated linoleic acid
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CRP	C reactive protein
HUVEC	Human umbilical vein endothelial cells
ICAM-1	Intracellular adhesion molecule-1
IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
MCP-1	Monocyte chemotactic protein-1
NF- κ B	Nuclear factor- κ b
NO	Nitric oxide
PDGF	Platelet-derived growth factor
PECAM-1	Platelet/endothelial cell adhesion molecule-1
sVCAM-1	Soluble vascular cell adhesion molecule-1
TIA	Transient ischemic attack
TNF- α	Tumor necrosis factor-alpha

INTRODUCTION

Ischemic stroke occurs when there is a sudden diminution in the blood supply to the brain. Endothelial dysfunction and atherosclerotic plaque formation play major roles in the narrowing of arteries supplying cerebral tissue. Observational studies suggest that linoleic acids may prevent the incidence of ischemic stroke. It has been hypothesized that these polyunsaturated fatty acids exert their effects by various mechanisms. In this paper the evidence regarding the probable role of conjugated linoleic acids in the prevention of ischemic stroke is presented based on the findings of observational studies, interventional studies in randomized, placebo-controlled clinical studies, *in vitro* cell cultures and animal models.

There is overwhelming clinical evidence which shows a direct correlation between saturated fatty acid intake and the incidence of cardiovascular and cerebrovascular diseases. Concurrently, there is much interest in the use of polyunsaturated fatty acids in the prevention of these conditions. Several studies have established an inverse relationship between polyunsaturated fatty acids intake, including linoleic acids, and the incidence of cardiovascular diseases. However, the impact of dietary intake of linoleic acids and the incidence of cerebrovascular events, including ischemic stroke, has been less extensively investigated.

OBSERVATIONAL EVIDENCE

One of the earliest case-control studies which attempted to link linoleic acid levels in the blood to the incidence of ischemic stroke was reported by Ricci and colleagues [1]. In this

study, the concentration of linoleic acid in red blood cell membranes was measured in 28 cases of recent ischemic events compared 56 age-matched controls. It was demonstrated that patients with ischemic stroke had significantly lower levels of linoleic acid compared with controls. Further evidence was provided by another case-control study which assessed linoleic acid content in subcutaneous adipose tissue in 10 recent cases of cerebral infarction and 10 matched controls [2]. These researchers showed that linoleic acid content in adipose tissue was 15% lower in ischemic stroke patients than in their matched controls.

A large prospective study screened and followed 7450 Japanese men and women between 40 and 85 years old for cardiovascular risk factors. After up to 9 years of follow-up, and 122 ischemic events, the study demonstrated that higher serum levels of linoleic acid was associated with a reduced risk of ischemic stroke [3]. It was postulated that this protection may be due to an effect of linoleic acid to lower blood pressure, reduce platelet aggregation and enhance erythrocyte deformation.

More recently, a study in Sweden followed 2313 middle-aged men for up to 32 years, with 412 cases of stroke or transient ischemic attacks during the period [4]. The investigators showed that a higher intake of linoleic acid protected against stroke and TIA. It was suggested that linoleic acid may be an appropriate long-term surrogate predictor for these cerebrovascular events.

EFFECTS ON MEDIATORS OF INFLAMMATION

Besides being a lipid disorder, it is now well established that atherosclerosis is a chronic inflammatory disease. Several pro-inflammatory mediators, known as cytokines, are pivotal in the initiation and maintenance of the inflammatory atherosclerotic state [5]. It is hypothesized that linoleic acid has its protective effect by attenuating the inflammatory responses which lead to the series of events leading to atherosclerotic plaque formation, and subsequently ischemic stroke.

Smedman and colleagues [6] conducted a randomized double-blinded placebo controlled study which included 53 men and women to determine whether conjugated linoleic acids supplementation affected markers of inflammation associated with the initiation and progression of atherosclerosis. The investigators showed that a 3-month supplementation with 4.2g/day (containing equimolar concentrations of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA isomers) significantly increased serum levels of the pro-inflammatory marker C-reactive protein. However, supplementation had no effect on levels of other inflammatory markers such as tumor necrosis factor- α (TNF- α) or sVCAM-1.

Another randomized, double-blind, parallel study by Tholstrup and colleagues [7] included 75 postmenopausal women who were given conjugated linoleic mixture, *cis*-9, *trans*-11 CLA or olive oil (placebo) over a 16-week period. Pro-inflammatory markers were measured at baseline and at the end of the study to determine the effects of the intervention. It was shown that the linoleic acid mixture significantly increased markers of inflammation including CRP, IL-6 and TNF- α ; but had no effect on markers of endothelial reactivity such as ICAM-1, VCAM-1 and MCP-1. On the other hand, supplementation with *cis*-9, *trans*-11 CLA had similar effects on inflammatory markers as the olive oil control.

At the molecular level, tumor necrosis factor- α (TNF- α) is a potent pro-inflammatory cytokine which plays a pivotal role in augmenting the inflammatory response via the intracellular transcription factor NF- κ B. De-Lima-Salgado and colleagues [8] showed that linoleic acid inhibited lipopolysaccharide (LPS)-induced TNF- α production in a mouse monocyte cell line. Cheng and colleagues [9] also demonstrated that conjugated linoleic acid inhibited the LPS-induced inflammatory response in a macrophage cell line (RAW 264.7). The decrease in inflammatory response was due in part to the significant decrease in NF- κ B binding to promoter regions for inflammatory mediators. There was dose-dependent inhibition of the LPS-induced up-regulation of iNOS and COX-2 expression, with subsequent decreased production of NO and prostaglandin E2. These findings were corroborated by another *in vitro* study which showed that CLA inhibited the expression of COX-1 expression and production of prostaglandin E2 and thromboxane A2, by attenuating NF- κ B nuclear translocation.

ANTI-ATHEROSCLEROTIC EFFECT OF LINOLEIC ACIDS AND MOLECULAR PATHWAYS

The initiation and propagation of atherosclerosis is a complex inflammatory process commencing with vascular injury, exposure of endothelium accompanied by adhesion and migration of monocytes into the intima. Oxidized LDL-cholesterol play a major role as macrophages are transformed into foam cells. The concerted interactions between transcription factors, intracellular signaling pathways and numerous pro-inflammatory cytokines, adhesion molecules and chemotactic molecules are vital to the formation of atherosclerotic plaques. The cascade of inflammatory events leading to initiation and progression of atherosclerosis involves the expression several mediators including intracellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and monocyte chemotactic protein-1 (MCP-1). MCP-1 has an important role in the migration, adhesion and infiltration of monocytes to the site of vascular injury and plays a key role in medial thickening at the site of atherosclerotic lesions. These events further promote atherosclerotic progression resulting in endothelial dysfunction and plaque formation [10].

To date, the clinical evidence is sparse regarding the hypothesis that CLA supplementation inhibits the initiation and progression of atherosclerosis. However, a randomized, double-blinded, placebo-controlled trial was conducted which involved 401 overweight or obese, but otherwise healthy subjects, given either 4g CLA (2.5g *cis*-9,*trans*-11 CLA, 0.6g *trans*-10,*cis*-12 CLA) or placebo for six months [11]. In this trial aortic pulse wave velocity was used as a surrogate measure for atherosclerosis, and biochemical assays were performed, including CRP to assess the effect of supplementation. At the end of the study the researchers found that pulse wave velocity did not change from baseline values in both groups, indicating that supplementation at this dose over the 6-month period had no effect on either initiation or progression of atherosclerosis. An additional finding was that *cis*-9, *trans*-12 conjugated linoleic acid had not effect of CRP levels; suggesting no anti-inflammatory effect.

On the other hand, several animal and *in vitro* studies were conducted to assess the effect of CLA and its isomers on established atherosclerosis, as well as to delineate the underlying molecular mechanisms involved. In an animal-based experiment by Kritchevsky and colleagues [12], rabbits were fed an atherogenic diet supplemented with CLA for 90 days. Compared to control animals, there was significant regression of established atherosclerosis in the arch and thoracic aorta by over 30% in CLA-treated animals. In yet another study, it was shown that apolipoprotein E knockout mice fed a high fat diet over 16 weeks, but given 1% ALA over the last 8 weeks, had not only regression of atherosclerotic plaque formation, but complete resolution compared with control animals [13]. It was also shown that there were significant decreases in aortic accumulation of macrophage and expression of macrophage scavenger receptor CD36 in CLA-fed animals.

Arbonés-Mainar and colleagues [14] conducted an *in vivo* study using apolipoprotein E knockout mice fed either *cis*-9, *trans*-11 CLA or *trans*-10, *cis*-12 CLA over 12 weeks. The investigators assessed atherosclerotic progression over the study period by measuring cross-sectional lesion and coverage area in the aortic root. It was demonstrated that *cis*-9, *trans*-11 CLA slowed the progression of atherosclerosis, whilst *trans*-10, *cis*-12 CLA accelerated progression.

In an *in vitro* experimental design using human macrophages incubated with *cis*-9, *trans*-11 CLA and linoleic acid for 48 hours there was significant increase in the expression of macrophage scavenger receptor CD36 [15]. This finding was in contrast to another *in vivo* study which showed otherwise [13]. A critical step in the initiation of atherosclerosis is the accumulation of lipids in macrophages, and Stachowska and colleagues [16] showed that *trans*-10, *cis*-12 CLA decreased lipid accumulation in macrophage and foam cell cultures.

Linoleic acid increased the platelet/endothelial cell adhesion molecule (PECAM)-1 and TNF- α -induced monocyte adhesion to human aortic endothelial cells [17]. These researchers also found that monocyte binding was enhanced by significant increase in E-selectin, vascular cell adhesion molecule (VCAM)-1, IL-8, MCP-1 and PGE2. In contrast, *cis*-9, *trans*-11 CLA reduced ICAM-1 and VCAM-1 expression on the surface of macrophages in culture [18]. It was also observed that this CLA isomer reduced the adhesion of macrophages to human umbilical vein endothelial cells (HUVEC). Similarly, using a HUVEC line, Lee and Vanden Heuvel [19] found that the less common *trans*-9, *trans*-11 isomer significantly reduced the expression of these atherogenic mediators, as well as E-selectin and P-selectin. These mediators are critically involved in the adhesion of monocytes to the site of vascular injury. Additionally, *trans*-9, *trans*-11 CLA decreased the adhesion of macrophages to the HUVEC cell line.

The complex pathways leading to arterial thickening, and subsequent occlusion, in atherosclerosis includes the incorporation of collagen into vascular intima-media. An *in vitro* experimental design using vascular smooth muscle cell lines from human aorta and coronary arteries showed that CLA isomers inhibited the platelet-derived growth factor (PDGF)-induced stimulation of collagen incorporation [20]. It was also shown that CLA inhibited the NF- κ B nuclear translocation, which could lead to the down-regulation of inflammatory pathways in the gene expression of chemokines and cytokines.

ANTITHROMBOTIC EFFECTS OF LINOLEIC ACIDS

The narrowing of the carotid artery by the formation of blood clots on ruptured plaques, which may break off and form emboli, also contribute to the dramatic reduction in cerebral blood supply and precipitate ischemic stroke. It has been hypothesized that linoleic acids may prevent cerebral infarction by modulating blood coagulation and fibrinolysis.

A clinical trial involving nine healthy young men, randomly assigned one of three diets enriched with stearic acid, oleic acid or linoleic acid, was conducted using a crossover design with 2-week intervention periods. There was at least 5 weeks washout between each intervention. In this study, by Hunter and colleagues [21], several factors for blood coagulation and fibrinolysis were measured at the start and end of each intervention period. The researchers found that fatty acid- enriched diets did not affect coagulability or fibrinolytic factors. However, it was shown that the linoleic-enriched diet significantly increased anti-aggregatory prostaglandin E1 binding to platelet membrane over the short-term.

In another clinical study [22], 17 healthy young women were randomly given either 3.9g/day conjugated linoleic acid supplement or sunflower oil (containing 72.6% linoleic acid, with no detectable CLA) for 63 days. The researchers found that short-term supplementation with CLA had no significant effect on platelet aggregation, prothrombin time, activated partial thromoplastin time or antithrombin III levels.

ANTIHYPERTENSIVE EFFECTS

Hypertension is a major risk factor for the development of ischemic stroke. Hypertension increases the likelihood of endothelial dysfunction and accelerates the events leading to atherosclerotic initiation and progression. The clinical evidence regarding the antihypertensive effects of linoleic acid is sparse. However, a randomized, placebo-controlled study involving 80 obese drug-naïve stage 1 hypertensive patients was conducted by Zhao and colleagues [23] to determine the effect of add-on conjugated linoleic acid with ramipril (37.5mg/day) over 8 weeks. The supplement, containing equimolar concentrations of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers, was shown to significantly enhance the blood pressure lowering effect of ramipril over the study period.

CONCLUSION

The clinical evidence provided by epidemiological and case-control studies suggest that a diet high in CLA may protect against ischemic stroke. Many pathophysiological factors are predictors of ischemic stroke, and inflammation is a major player in the etiology of atherosclerosis. Interventional clinical studies showed that CLA supplementation over the short term actually increased the expression of inflammatory markers, which should enhance molecular events leading to atherosclerosis. On the contrary, *in vitro* studies show that CLA inhibited inflammation in stimulated cell cultures.

The single clinical trial showed that CLA supplementation had no effect on atherosclerotic progression over six months. However, animal studies show isomer-specific

regression and complete resolution in established atherosclerosis. With regard to the involvement of monocytes/macrophages in the initiation of atherosclerosis, *in vitro* studies indicate that CLA modulates the expression of chemotactic mediators and adhesion molecules.

Observational evidence indicates that higher dietary intake of conjugated linoleic acids may reduce the risk of ischemic stroke. However, the findings from clinical trials and laboratory studies are mixed and do not clearly delineate mechanisms by which CLA may be acting to provide protection against ischemic stroke.

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Chapter 51

FROM KNOWLEDGE TO PRACTICE: SUSTAINING COMMUNITY ENGAGEMENT IN SECONDARY STROKE PREVENTION

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ABSTRACT

Despite advances in pharmacological and surgical interventions, patients' participation in therapeutic lifestyle modification after a minor stroke has been emphasized as the cornerstone of secondary stroke prevention. Therefore, minor stroke sequelae are not only a medical matter but also a self-management matter. Patients who have just experienced a minor stroke need to be equipped with self-management knowledge and skills to effectively participate in secondary prevention. This chapter presents a community initiative of an evidence-based self-management educational intervention complementing therapeutic intervention for secondary stroke prevention. Essential components for knowledge transfer from professional care providers to patients in community setting are discussed. Finally, this chapter makes an attempt to explore opportunities and challenges for sustaining community engagement in secondary stroke prevention for minor stroke patients.

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INTRODUCTION

Transient ischaemic attack or minor stroke (herein ‘minor stroke’) continues to be one of the most under-estimated developmental stroke syndromes. It is a syndrome characterized by the sudden onset of discrete neurological symptoms that resolve completely within 24 hours without structural brain damage (Roger, Go, Lloyd-Jones, et al., 2011). Symptoms include numbness or weakness in the face, arm, or leg on one side of the body; slurred speech or difficulty in understanding speech; trouble seeing in one or both eyes; dizziness, loss of balance and coordination, and difficulty in walking (National Institute of Neurological Disorder and Stroke, 2011). In Hong Kong, the incidence of minor stroke from 1999 to 2007 is stable at around 8% (Chau et al., 2011). Over the 2009-2010 period there were 2,008 minor stroke patients discharged from hospitals in Hong Kong (Hospital Authority, 2011). Minor stroke has always been considered as a harbinger of ischaemic stroke (Rothwell, et al., 2006; American Heart Association, 2011). The Oxford Vascular Study found that after a minor stroke, the risk of subsequent ischaemic stroke was 8%-12% at seven days, 11%-15% at one month, and 17% at three months (Coull, et al., 2004). There is a 50% stroke risk in the first year and up to a 35% stroke risk 3-5 years after a minor stroke (Ho, 2002; Klein-Ritter and Wasserman, 2001). Minor stroke therefore is an urgent medical matter. The public in general, and minor stroke patients in particular, need to be educated to seek medical attention urgently for timely investigations and stroke prevention treatment.

Apart from uncontrollable risk factors such as age, ethnicity, having a family history of stroke, and previous minor strokes, there are two types of controllable risk factors: (1) medical factors, such as hypertension, atrial fibrillation, carotid artery disease, diabetes and hyperlipidaemia, and (2) lifestyle factors, such as physical inactivity, unhealthy diet, alcohol and tobacco use (National Stroke Association, 2011). Both types of controllable stroke risk factors can be managed: the former can be treated and the latter can be changed. For secondary stroke prevention to be effective, evidence from the National Stroke Association (2011) suggests that lifestyle modification is as equally important as pharmacological and surgical interventions for tight control of risk factors. Secondary stroke prevention can therefore be seen not only as a medical matter, but also a self-management matter since it requires modification of lifestyle habits, which heavily depend on the individual patient’s commitment and participation. However the patients’ participation in lifestyle modification is sub-optimal, especially after hospital discharge (Berra, 2010). The challenges for health care providers are to find ways to empower these patients to take on responsibility and to actively participate in self-management for secondary stroke prevention.

This chapter addresses (1) the paradigm shift needed in patient education for secondary stroke prevention; (2) the development of a community-based self-management education programme; (3) research evidence on the effectiveness of self-management educational interventions for patients with minor stroke; (4) the incorporation of research evidence into community practice and (5) ways of sustaining community engagement in secondary stroke prevention.

SELF-MANAGEMENT APPROACH IN SECONDARY STROKE PREVENTION: A PARADIGM SHIFT

It is commonly understood that treatment and care for patients does not end at hospital discharge. Very often, patients with chronic illness require longer follow-up care or post-discharge intervention that goes beyond the hospital boundary. Patients with minor stroke, due to the reversibility of neurological symptoms, tend to under-estimate the possibility of a subsequent stroke and its long-term impact on their health. Because of this under-estimation, patients with minor stroke warrant a tailor-made stroke prevention programme.

Table 1. Comparison of traditional patient education and self-management education.

	Traditional patient education	Self-management education
What is taught?	Disease-specific knowledge and information. Technical skills related to disease management such as blood pressure monitoring.	Knowledge and information to gain understanding related to disease management. Generic skills, such as problem-solving, goal setting and action planning, making choices, and resource utilization related to living with the disease.
What is the goal?	Compliance with treatment regimen with the behavioural changes taught to the patients to improve clinical outcome.	Increased self-efficacy to participate in day-to-day disease management for improved clinical outcome.
What is the underlying assumption?	Improved disease-specific knowledge creates behavioural change, which in turn produces better clinical outcomes.	Greater patient confidence in his/her capacity to make life-improving changes, which in turn improves clinical outcomes.
How is behaviour changed?	External motivation Health professional sets behavioural goals and actions for patient to follow.	Internal motivation Patients gain understanding and confidence to make informed choices for their own behavioural goal attainment.
What is the relationship between patient and health professionals?	Professionals are the experts and represent authority to tell patients what to do. Patients are the recipients.	Professionals are experts about the disease and patients are experts about their lives and what works for them. Patients and professionals share information and decision-making.
What is the teaching process?	Didactic. Professionals teach/provide information, patients listen and ask questions.	Small group interaction. Professionals as facilitators for peer group discussion, experience sharing and reflection.
Who is responsible for outcomes?	Professionals.	Shared responsibility between professionals and patients for solving problems and outcomes.

(Bodenheimer, et al., 2002)

A community-based project aimed at keeping minor stroke patients informed of the realities of their neurovascular changes and at involving them in taking responsibility to actively participate in prompt preventive measures to prevent/reduce the devastating consequences of a stroke was initiated in 2003. The key issue addressed was not whether minor stroke patients manage their illness, but *how* they manage.

This Project adopted an empowering partnering approach to care, in contrast to the traditional paternalistic approach. An empowering partnering approach encompasses two elements:

First, self-management education equips patients with knowledge about the disease as well as technical and problem-solving skills for day-to-day health monitoring and disease management. The theoretical framework underpinning the shift to self-management education is from self-efficacy theory (Bandura, 1977), which refers to the confidence level that a person needs to carry out a behaviour necessary to reach a desired outcome.

Through self-management education, patients gain understanding and confidence to accomplish new behaviours for goal attainment and subsequent desirable outcomes. Table 1 compares the differences between the traditional educational approach and the self-management approach (Bodenheimer, et al., 2002).

Second, a patient-professional partnership values the joint collaboration, where professionals bring knowledge and expertise about therapeutic goals and disease management options, while active and prepared patients bring expertise on their lives, their daily experiences and what will work for them (Funnell and Anderson, 2004).

A collaborative empowering partnering approach facilitates informed patients to accept shared responsibility with their health care providers for managing their health conditions and for solving daily health-related problems with informed choices, instead of passively following a health professional's prescription.

THE DEVELOPMENT OF A SELF-MANAGEMENT EDUCATIONAL PROGRAMME FOR MINOR STROKE PATIENTS

For an empowering and partnering approach to care, two important items in developing a self-management educational programme are the programme content and the teaching process.

The programme content must be able to prepare minor stroke patients with the necessary information to participate in secondary stroke prevention. Bodenheimer, et al., (2002) suggested that the information should include medical information about the nature of stroke, its clinical manifestations, warning signs, medical and therapeutic lifestyle interventions for secondary stroke prevention as well as social information, e.g., the availability of relevant local resources to support informational and self-management needs. The content of our specific Programme was based on the National Stroke Association guidelines (2011) on therapeutic lifestyle changes for secondary stroke prevention, with consideration for and adjustment of cultural-specific aspects for Hong Kong Chinese. Table 2 shows the self-management objectives and key components in the Programme.

The teaching process aimed to effectively empower patients to participate in secondary stroke prevention. In this Programme, the self-management educational intervention was

conducted in 2-hour small group sessions of 10 to 12 minor stroke patients, held once a week for 8 weeks in initial implementation and modified to 5 weeks in subsequent community practice.

Table 2. Self-management objectives and key components.

Self-management objectives	Key components
Discussion of experience of the minor stroke and self-management for secondary prevention.	Ice-breaking session for initiating a collaborative relationship among facilitators and participants. Participants sharing their individual experiences of and their feelings about the minor stroke. Participants explore the importance of self-management for secondary stroke prevention.
The facts about stroke: nature of stroke, its clinical manifestations, consequences, and risk factors.	Participants begin building knowledge about what happened to their body, why and how it happened, and what possible consequences could result from a stroke. Recognising the warning signs of stroke, the importance of reaching emergency medical facilities and action to call 999 (equivalent to 911 in USA) in the case of a suspected stroke. Recognising common stroke risk factors that are related to self-management for secondary prevention.
Current stroke management and secondary prevention.	Continuing to build knowledge of current therapeutic preventive intervention for minor stroke. Understanding of how self-management can complement therapeutic treatment is explored and discussed.
Understanding medication regime and importance of adherence.	Understanding therapeutic goals for tight risk factors control. Reinforce the importance of medication adherence and explore ways to enhance medication adherence. Identification of any difficulty in following the doctor's prescription, problems encountered and possible solutions.
Stroke prevention through adopting a healthy lifestyle.	Reinforce knowledge on behavioural risk factors. Recognising the participant's own stroke risk factors and risk behaviours in lifestyle. Verbalising individual goals and naming of actions required for preventing stroke recurrence. Exploring problem-solving to overcome hurdles.
Eating a healthy diet.	Building knowledge about the American Heart Association and National Stroke Association guidelines on healthy diets. Educating participants on how to make healthy choices when shopping for food or dining out. Exploring healthy cooking methods (attention paid to culturally relevant Chinese cooking). Exploring the benefits and cautions of eating herbal soup or medicated meals.
Establishing a regular exercise habit.	Suggesting participants to explore ways to initiate / enhance physical activities. Exploring individual preference for exercise pattern, length of exercise & pace of exercise. Correcting misconceptions about exercise. Coaching participants to practise self health monitoring for desirable exercise intensity and duration.
Putting thoughts into actions.	Consolidating goal setting and action plans for participating in lifestyle modification and self-management. Participants share personal insights gleaned from the experiential learning, and acknowledge their individual accomplishments.

(Sit, et al., 2007)

Experienced community care professionals (registered nurses in the initial implementation and registered social workers for subsequent implementations and networking) ran the

Programme as facilitators. The enhancement of self-efficacy was a key component guiding the self-management education process. Patients' self-efficacy beliefs can be enhanced through performance accomplishments, vicarious experience, verbal persuasion and physiological information all of which were part of the small group interaction (Lorig and Holman, 2003).

Performance Accomplishments

Learning and practising appropriate behaviours are considered to be the most potent efficacy-enhancing strategy—the experience of success (feeling of mastery) enhances self-efficacy, while regular failure decreases self-efficacy. In the Programme, facilitators acknowledge attempts and emphasize favorable behavioural changes made by the patients. Experience with behaviour and the attributions of success are an important source for the development of self-efficacy.

Vicarious Experience

Observing others' successful performance is another source of self-efficacy. They can serve as realistic positive role models and sources of inspiration by supplying information/tips about making a success of specific health behaviours. Some participants were sensitive to observed information when they themselves were inexperienced with the specific kind of behavioural change. They used observed indicators from which they could measure their own capabilities and then estimate their own possible success.

Verbal Persuasion

Verbal persuasion is the most often used source of self-efficacy. It is effective when it involves encouraging participants to attempt a little more than they are currently doing. Verbal attempts to convince people that they have the ability to perform a little more were frequently used by the facilitators to encourage participants in setting goals and devising an action plan. Also peers (other minor stroke patients in the group) can influence a participant who is reluctant to initiate a course of action (Gonzales, Goepfinger and Lorig, 1990). When participants were convinced of their abilities, they were more prone to persevere and not easily give up.

Interpretation of Physiological Information

Information about physiological parameters can also influence a participant's motivation and perseverance to initiate and continue specific self-management behaviours. In the Programme, participants were taught to take their pulse rate and to use the Borg Perceive Exertion Rating for assessing their own exercise tolerance. Such information could motivate patients to exercise at a desirable intensity and duration as a behavioural goal attainment.

Similarly, understanding high blood pressure as one of the stroke risk factors and the subsequent targeting of blood pressure range for tight control could reinforce the patients' understanding of the importance of and participation in daily self blood pressure monitoring, record keeping and medication adherence.

ESTABLISHING EVIDENCE-BASED PRACTICE IN COMMUNITY-BASED SECONDARY STROKE PREVENTION INITIATIVES FOR PATIENTS WITH MINOR STROKE

In order to establish evidence-based practice, a research study was conducted to examine the effectiveness of the Programme in (1) improvement in knowledge about stroke, (2) improvement in self-health monitoring practice, (3) behavioural change in adopting healthy lifestyle habits for stroke prevention, and (4) the reduction in stroke risk factors.

Method

The study employed a quasi-experimental design with participants being randomized by time slot to either a control group (CG) or an intervention group (IG). The CG received conventional medical follow-up while the IG received an eight-week community-based Secondary Stroke Prevention Programme in addition to the conventional medical follow-up. Outcome data were collected at three time points: before the intervention (T0), one week after (T1) and three months after (T2) the completion of intervention. Participants with the following characteristics were recruited: aged 18 or above, diagnosed to have had minor stroke by doctors, medically stable, independent in activities of daily living, cognitively intact, able to communicate in Cantonese, discharged from hospital and currently living in the community. Those with surgical intervention pending, with congenital cerebrovascular abnormalities or with stroke-like syndromes from other causes, were excluded. Data were obtained from face-to-face interviews using a structured questionnaire which included four sections: (a) sociodemographic profile, (b) lifestyle habits including smoking, drinking, self-health monitoring practice, exercise and dietary habits, (c) medication compliance, and (d) knowledge about stroke warning signs and risk factors. In addition, individual participants' physical parameters of stroke risk factors were screened, which included blood pressure, body mass index and fasting blood lipid (total cholesterol and triglyceride by *Accutrend^R* point-of-care device).

Results

The study recruited 190 participants. One hundred and forty seven completed the study, of whom 77 were from the intervention group and 70 from the control group. Twenty eight participants (25%) from the intervention group and 16 participants (19%) from the control group dropped out. Reasons for drop-out included: re-hospitalization, away from Hong Kong seeking medical treatment in mainland China, travelling overseas, and failure to contact. Also

participants in the intervention group who did not attain 80% attendance were counted as drop-outs. Comparison between completers and drop-outs showed no statistical significant difference in demographic characteristics.

Table 3. Demographic characteristics between participants in the intervention group and control group.

Demographic characteristics	Intervention group (n=107)	Control group (n=83)	P value ⁽¹⁾
	Frequency (%)	Frequency (%)	
	Age group (years)		
Below 45	4 (3.7)	6 (7.2)	0.109
45-64	46 (42.6)	33 (39.8)	
65-74	47 (43.5)	27 (32.5)	
75 or above	11 (10.2)	17 (20.5)	
	Sex		
Male	55 (49.5)	50 (62.5)	0.412
Female	52 (50.5)	33 (37.5)	
	Marital status		
Married	79 (73.8)	68 (82)	0.412
Separated/single	18 (16.9)	10 (12)	
Widowed	10 (9.3)	5 (6)	
	Educational level		
No formal education	10 (9.3)	12 (14.5)	0.438
Primary school level	33 (30.8)	29 (34.9)	
Secondary school level	52 (48.6)	33 (39.8)	
Post secondary level	12 (11.2)	9 (10.8)	
	Residential status		
Live alone	16 (15)	4 (4.8)	0.013
Live with family	91 (85)	79 (95.2)	
	Occupational status		
Working	15 (14)	14 (16.9)	0.878
Unemployed	10 (9.3)	7 (8.4)	
Homemaker	28 (26.2)	18 (21.7)	
Retired	54 (50.5)	44 (53)	
	Extended ADL ⁽²⁾ status		
By self	58 (54.2)	35 (42.2)	0.1
Spouse	33 (30.8)	34 (41)	
Children	7 (6.5)	8 (9.6)	
Maid domestic helper	9 (8.5)	6 (7.2)	
	Family history of stroke		
No	67 (62.6)	62 (74.7)	0.117
Yes	40 (37.4)	21 (25.3)	

⁽¹⁾ P value was calculated with chi-square test

⁽²⁾ ADL denotes activity of daily living

(Sit, et al., 2007, p.277)

Key findings of the study are reported elsewhere (Sit, et al., 2007). Briefly, demographic characteristics of the participants showed that around 47% of them were aged below 65 years. This percentage indicates that stroke and ischaemic cerebrovascular events are not limited to older people. Over 50% of the participants had secondary school education or above. As shown in Table 3, demographic characteristics between participants in the intervention group and control group were compared and no statistical significance was found between the groups except that more participants in the IG lived alone than those in the CG ($p=0.013$). Regarding the presence of stroke risk factors, nearly 40% of the participants had hypertension. Around 32% of them were overweight, 27% and 18% had suboptimal blood triglyceride and total cholesterol level respectively. Regarding behavioural risk factors, only a small percentage were current smokers and alcohol drinkers, but a majority of the participants (63%) were physically inactive. Our findings are comparable with the local population statistics although the percentage of physically inactive people was higher (Centre for health Protection, 2011).

Intention-to-treat analysis was performed to examine the changes in outcome variables over the three-time points. Missing data were replaced with baseline values. Table 4 shows the outcome data at baseline, post one-week and three-month follow-up. Regarding knowledge level, participants in the IG showed significant improvements in correctly identified stroke warning signs ($p<0.001$) and emergency treatment seeking response in case of a suspected stroke ($p<0.001$), the latter had a 4-fold increase in correct responses. In behavioral risk factors, there was significant reduction in the intake of salted preserved food ($p=0.004$) and foods with high saturated fat content ($p=0.032$). The percentage of walking exercise participation in the IG group had stable performance over the 3 data collection time points ($p=0.98$), while the CG group participants had a 23.4% decrease ($p=0.021$). In self-management behavioural change, there was a significant improvement among IG participants in adherence to the medication regimen ($p<0.001$) and in self blood pressure monitoring ($p<0.001$). There was a 50% increase in IG participants who regularly monitored their own blood pressure.

Regarding physiological risk factors, a reduction in fasting triglyceride level was detected over the 3 months data collection period in both groups. The IG however showed a significant statistical reduction ($p<0.001$) where the CG did not. It was also noted that the BMI in the CG showed a statistically significant increase ($p=0.034$) while the IG had slight but not statistically significant decrease at the 3-month follow-up ($p=0.088$). It may be possible that participants in the IG made more appropriate lifestyle changes than the control group. No significant change was found among other physiological risk parameters, such as blood pressure, total cholesterol level and blood sugar level.

Table 4. Outcome data at baseline, post one-week and three-month follow-up.

			T ₀	T ₁	T ₂	Friedman	P
			mean (sd)	mean (sd)	mean (sd)		
Stroke knowledge							
Knowledge on stroke warning signs ^a							
	Intervention		70.87 (2.74)	83.95 (2.07)	81.88 (2.34)	23.38	<0.001
	Control		71.69 (3.23)	76.51 (2.92)	74.70 (3.05)	2.45	0.293
Knowledge on stroke risk factors ^{a, d}							
	Intervention		85.66 (1.45)	87.18 (1.31)	84.86 (1.66)	2.82	0.244
	Control		77.02 (2.27)	77.75 (2.18)	75.26 (2.22)	0.63	0.731
Lifestyle modification							
Medication compliance ^{b, g}							
	Intervention		3.34 (0.86)	3.75 (0.48)	3.45 (0.74)	20.71	<0.001
	Control		3.15 (0.93)	3.17 (1.04)	3.21 (1.03)	1.25	0.293
Consumption of salted preserved food ^c							
	Intervention		2.03 (0.87)	1.82 (0.79)	1.83 (0.76)	11.2	0.004
	Control		2.13 (0.86)	2.01 (0.81)	2.01 (0.87)	3.94	0.14
			percentage	percentage	percentage	Cochran's Q	P
Stroke knowledge							
Treatment seeking response in case of stroke ^h							
	Intervention		18.3	100	93.6	106.86	<0.001
	Control		16.3	43.8	31.9	28.53	<0.001
Self-health monitoring practice							
Participated in self BP monitoring ^{e, i}							
	Intervention		55	74.3	82.6	34.68	<0.001
	Control		39.8	48.2	43.4	3.7	0.157

			percentage	percentage	percentage	Cochran's Q	P
Lifestyle modification							
Participation in walking exercise ^j							
	Intervention		78.9	78.9	77.1	0.051	0.975
	Control		72.3	63.9	55.4	7.697	0.021
Eating thick poultry bone soup ^f							
	Intervention		31.2	23.9	21.1	6.867	0.032
	Control		30.1	22.9	25.3	2.333	0.311

^a (0-100) the higher score indicating better level of knowledge

^b (1-4) the higher score indicating better medication compliance

^c (1-5) the lower score indicating less frequent consumption of salted preserved food

^d Significant difference between intervention and control group at T₀ (Mann-Whitney U, p=0.002)

^e Significant difference between intervention and control group at T₀ (Chi-square, p=0.042)

^f A common type of Southern Chinese cooking which contains high saturated fat content

^g Significant difference between intervention and control group at T₁ follow-up (Mann-Whitney U, p=0.004)

^h Significant difference between intervention and control group at T₁ (Chi-square, p<0.001) & T₂ (Chi-square, p<0.001)

ⁱ Significant difference between intervention and control group at T₁ (Chi-square, p<0.001) & T₂ (Chi-square, p<0.001)

^j Significant difference between intervention and control group at T₂ (Chi-square, p<0.001)

(Sit, et al., 2007, p.279)

TRANSLATING RESEARCH EVIDENCE INTO COMMUNITY PRACTICE IN SECONDARY STROKE PREVENTION FOR MINOR STROKE PATIENTS

That the community-based self-management educational intervention for secondary stroke prevention was effective in improving minor stroke participants' stroke knowledge, dietary and exercise lifestyle modification, self-management practices in blood pressure monitoring and adherence to a medication regimen is supported by the research evidence. Slight improvements in blood triglyceride level and BMI were noted, but the 3-month follow-up period might not be long enough to determine whether such improvements could eventually be able to meet the target values for tight risk factors control. The research experience and intervention protocol (educational programme contents and teaching process) had provided valuable groundwork and recommendations for subsequent large scale community implementation.

The followings highlight some essential experiences for knowledge transfer from professional care providers to patients in community-based educational programme.

Promoting Patients' uptake and Participation

Patient educational intervention is an important component of secondary stroke prevention but patients' uptake of and participation in such programmes are often below the recommended levels, especially after hospital discharge (Davies, et al., 2010). This problem prompted community practitioners to network with nurses in acute settings for programme promotion and making formal referral. Community practitioners' motivational communications via telephone calls were also found to be effective in increasing patients' uptake and participation in the community-based educational Programme. Our experience is in keeping with the conclusion from Davies, et al., (2010) systematic review.

Timing

It is important to capture the right time to inform and motivate minor stroke patients to participate in secondary stroke prevention. Educational intervention given soon after hospital discharge would be appropriate. After hospitalization, patients are away from professional care and face the challenges of managing their unpredictable health condition and making daily decisions on ways to maintain health. Introduction to knowledge through a community educational programme at this time may be compatible with the patients' learning needs, so they may then be more receptive to the information.

Knowledge and Skills Transfer

Self-health monitoring is an integral part of self-management for secondary stroke prevention. After attending the Programme, minor stroke participants' increased knowledge of hypertension as an important stroke risk factor was translated into an increase in their self

blood pressure monitoring practice. This reflects the importance of not only transferring knowledge, but also transferring self-management skills which included supervised hands-on practice during the Programme, self-practice at home, feedback and group sharing in subsequent sessions of the Programme.

Equally important is the transferring of problem-solving skills to enhance the patients' ability to deal with obstacles in initiating or maintaining a healthy lifestyle practice (Bodenheimer, et al., 2002). For example, advice on healthy eating is a culturally specific topic, so guidelines recommended by Western countries may not match the Cantonese eating style, food availability in local markets and affordability for individual patients. Therefore, to introduce dietary behavioural change, the Programme did not demand that participants follow specific menus but introduced them to making informed choices for deciding a stroke risk-reduction eating pattern that suited their own preferences and affordability. In Chinese culture, the dining table is not only for eating but also for socializing. Meals are served with shared dishes among a group of family members or friends. Problem-solving skills are deemed necessary for patients to deal with hurdles encountered when sharing eating with others, especially in the collective culture within the Chinese community.

Experiential Learning and Feedback

Self-management educational intervention emphasizes 'know-how' knowledge which is better learned through doing and experiencing (Price, 1993). The Programme engaged participants in short-term goal setting and action planning. The participants were asked to identify their own behavioural risk factors which they wanted to modify, addressing them one at a time, then to set short term behavioural goals and devise an action plan. In the week proceeding to the next session, participants had the opportunity to implement their action plan at home. The participants were asked to report back their home practice performance in the next group session. The facilitator gave feedback on the consistency/discrepancy between their plan and their reported actual behavior. Participants were given time to reflect on facilitating factors or barriers to behavioural change and to explore problem-solving strategies. The Programme affirmed the importance of experiential learning experience in self-management education and recognized the importance of individualized regimes developed from personal experiences and perceptions of what worked for them (Sit, et al., 2007)..

Peer Support

Heisler, et al., (2010) suggested that peers facing the same health behavioural or self management challenges could provide better reciprocal peer support for patients to manage their conditions. In the Programme, small group sessions with 10-12 participants in each group provided a platform for peer interaction and support. Each self-management education session opened with 15 minutes of experience sharing and closed with a word of commitment for goal attainment from each individual participant. The groups were conducted in a supportive and non-judgmental atmosphere to strengthen confidence and motivation. Experiences of successful goal attainment shared among peer participants provided sources of

inspiration to other participants by supplying information about the degree of difficulty of a specific behavioural change and strategies to overcome the difficulty. Also, through group interaction, participants themselves were able to realize how much they could offer and receive in the reciprocal support relationship. Experience from the Programme implementation suggested that an effective way to activate patients was to provide them the opportunity to both help and be helped through small group interaction.

SUSTAINING COMMUNITY ENGAGEMENT THROUGH TRANSDISCIPLINARY COLLABORATION

Since the initial implementation in 2003, the Programme has grown considerably with expanded networking with other health service providers. Similar to other chronic diseases, most stroke risks have behavioural factors. As discussed previously, therapeutic lifestyle modification is not a pure medical science, it involves behavioral and social sciences. Sustaining community engagement in secondary stroke prevention must draw together the strengths from health and social sectors with different knowledge and skills. This requires crossing the professional boundaries by combining the expertise of two or more professional fields to achieve a more holistic and sustainable community engagement in chronic disease management.

Transdisciplinary approach in Patient Empowerment

There are different types of cross-disciplinary approaches in practice for chronic disease management. The transdisciplinary team approach, which this Programme adopted, refers to professionals *working jointly using the same framework of care and sharing information* that draws together discipline-specific knowledge, concepts and intervention approaches to address mutually agreed aims and patient outcomes. It can be contrasted with the interdisciplinary approach where professionals *jointly work together* to address commonly agreed aims and patient outcomes; and the multidisciplinary team approach where professionals in different disciplines work *independently and sequentially* to address their own disciplinary-specific aims and patient outcomes (Gowdy, 2006).

A major challenge in the transdisciplinary approach to care is priority setting (Gibson, et al., 2002). A discipline-specific approach to priority setting has been found to be insufficient because it is not grounded in actual patients' needs and self-management problems as well as the social circumstances where self-management takes place. Therefore, in priority setting for self-management education, a synthesis of both health-specific and generic approaches to care is needed. For example, in programme planning, professionals of health and social disciplines join efforts and share expertise to develop self-management intervention on medication adherence. Health specific objectives include reinforcing the importance of medication compliance for secondary stroke prevention (e.g., the dos and don'ts for taking medication safely and correctly). Behavioural objectives include introducing generic problem-solving skills on *how to* enhance medication compliance relevant to an individual patient's work and life circumstances (e.g., how to minimize barriers and facilitate adherence, how to

communicate with their doctor when there is a health concern and how to find local community resources for professional advice). Through sharing of preliminary ideas among people from different disciplines, collaborative goals are mutually agreed and information delivery formats structured.

Another challenge in the transdisciplinary approach is interprofessional knowledge exchange. It is generally understood that putting professionals from different disciplines in the same classroom and having them teach together may not meet the challenges of cross-fertilization and knowledge exchange. In this project, interactive workshops and project team working groups have shown promising facilitation for information/knowledge exchange among community practitioners of different professional disciplines. By working collaboratively together and shared responsibility in patient outcomes, linkages between professionals were fostered. This could be expected to have resultant benefits for the transdisciplinary approach of care in community. Experience revealed from this Project highlighted knowledge and skills exchange among community practitioners from health and social disciplines. That is the synthesis of health related knowledge and motivational skills to bring about patients' behavioural change for lifestyle modification and taking up self-management responsibility for secondary stroke prevention.

Hospital-Community Interfacing

In chronic disease management, failure to meet self-management needs has long been a prime source of hospital readmission and patients/family carers' dissatisfaction. For minor stroke patients, the consequences could be more devastating because without adequately preparing and engaging these patients to self-manage for secondary stroke prevention, they could be at higher risk for another more serious cerebrovascular event. It has been generally expected that hospital health professionals are responsible for ensuring that patients are properly informed and adequately prepared to manage themselves for secondary stroke prevention. However, this may not be a realistic expectation in this era of short length of hospital stay, and therefore more responsibility for meeting self-management needs must now be shouldered in the community (Worth, et al., 2000).

To sustain community engagement in secondary stroke prevention, the need for more effective hospital-community interfacing has already been recognized by practitioners in health care and social work (Department of Health, 2008). Community practitioners, sometimes external to the mainstream health care sectors, have a key role in establishing conditions for the creation and dissemination of knowledge/information in self-management educational intervention and chronic disease management.

Four levels of service interfacing can be identified (Table 5). Linkage among service providers can be facilitated through initial networking (for example, information exchange on joint conference or health initiatives; shared newsletters and mailing lists) and then subsequently establishing formal linkage among service providers (for example, setting up formal chronic illness management platforms for communication among service providers from different sectors). Service interface at this level encompasses interfacing workflow, referral mechanisms, joint pre-discharge health talks and other post-discharge interfacing services and programmes.

Table 5. Levels of hospital-community service interfacing.

Level of interface	Components of interface		
	Resource	Governance and operation	Service
Network	Shared information	Nil	Nil
Linkage	Shared information	Overseeing committee	Single and multiple projects
Partnership	Shared information and staff	Shared governance	Current and new projects/ services
Integration	Shared information, staff and funding	Shared leadership and governance	Expanded and new services

(Chan, 2010)

Advancing the development to the hospital-community partnership level requires shared patient data (electronic database), interprofessional education, and shared responsibility of patient outcomes. However, Thomasgard, et al., (2004) identified challenges to service interfacing at partnership and integration levels, which are the existing differences inherited within the hospital and community care sector. These include different organizational goals and funding priorities, different approaches to protecting patient data and confidentiality, varying ethnocultural backgrounds among service providers from different sectors, and the diverse structure, size and resources associated with various fields of practice. Hence, in keeping with the paradigm shift in hospital-community service interfacing, a core function of professionals, administrators and policy makers is to build community capacity to enhance health care and chronic disease management.

CONCLUSION

The chapter presented a transdisciplinary community-based secondary stroke prevention project undertaken to promote the application of research findings that enhanced minor stroke patients' engagement in self-management for secondary stroke prevention. It highlighted the fact that patient education should go beyond the traditional medical oriented 'know-what' knowledge to include clinical and social relevant 'know-how' knowledge and self-management skills. This requires joint efforts and shared expertise among community practitioners from different disciplines to synthesize knowledge and skills for preparing and engaging patients' participation in self-management. To sustain community engagement for secondary stroke prevention, transdisciplinary care approach and hospital-community service interfacing as well as their related challenges were also discussed.

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Chapter 52

RISK OF STROKE AND DEATH WITH ANTIPSYCHOTICS IN DEMENTIA PATIENTS

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ABSTRACT

Behavioral and psychological symptoms are common in demented patients. They are a common cause for increased morbidity and mortality in these patients. These behaviors and symptoms contribute significantly to the direct and indirect costs of caring for patients with dementia. Antipsychotic medications have shown modest efficacy in the treatment of these behaviors. However, their use in demented patients has generated controversy due to the increasing recognition of the side effects especially cerebrovascular adverse events (CVAEs) and death. In this chapter we review the data on antipsychotic medications causing cerebrovascular adverse events (CVAEs) and death in elderly demented patients.

INTRODUCTION

Behavioral and psychological symptoms of dementia (BPSD) are defined as a heterogeneous range of psychological reactions, psychiatric symptoms, and behaviors, which may be disruptive, unsafe and impair the care of the patient in a given environment [1]. BPSD is common in demented patients and their prevalence tends to increase with the progression of the illness [2]. BPSD has a profound impact on both the patients and their caregivers and is associated with increased morbidity, cost of care and mortality [2]. Antipsychotic medications are commonly used to treat BPSD although they have modest efficacy and there is greater recognition of their side-effect profile [3-9].

The health regulatory agency in Canada was the first to raise concerns about the association of risperidone with cerebrovascular adverse events in clinical trials of elderly patients with dementia in 2002 [10]. This was followed by the US Food and Drug Administration (FDA) in 2003, publishing warnings about cerebrovascular adverse events and requiring changes in the prescribing information for risperidone [11]. In 2004, the European Agency for the Evaluation of Medicinal Products (currently known as the European Medicines Agency) issued public advice about the increased risk of cerebrovascular adverse events and mortality in elderly patients with dementia receiving olanzapine [12]. Again in 2004, the United Kingdom's Committee on Safety of Medicines (now known as the Commission on Human Medicines) advised prescribers that risperidone and olanzapine should not be used to treat BPSD because of clear evidence of an increased risk of stroke [13]. In 2005, the FDA published a warning that the treatment of behavioral disorders in elderly persons with dementia with atypical (second-generation) antipsychotics is associated with increased mortality [14]. There were a total of 17 placebo-controlled trials investigating olanzapine, aripiprazole, risperidone or quetiapine in elderly persons with dementia and concomitant behavioral disorders. Of the 17 trials, 15 showed numerical increases in mortality in the group receiving drug treatment as compared with the group receiving placebo. There were a total of 5106 patients that were enrolled in these studies and analyses have demonstrated an approximate 1.6 to 1.7-fold increase in mortality in these studies. Most of these deaths were found to be due to either heart-related events (eg, heart failure, sudden death) or infections (mostly pneumonia). The FDA asked the manufacturers of these drugs to include a Boxed Warning in their labeling to describe this risk and note that these drugs are not approved for the treatment of this indication. In addition to olanzapine, aripiprazole, risperidone and quetiapine, the atypical antipsychotic medications clozapine and ziprasidone and a combination product containing olanzapine and fluoxetine were included in the warnings. In 2008, the FDA extended the warning to also include conventional antipsychotic drugs [15].

In this chapter, we review the risk of CVAEs and death with antipsychotic medications when used to treat elderly patients with dementia. Using the current data we provide evidence based treatment recommendations for these behaviors. We organized the chapter into the risk of CVAEs followed by risk for death. Data for each of the events were further subdivided into randomized controlled trials, meta-analysis and other trials.

1. CEREBROVASCULAR ADVERSE EVENTS (CVAEs)

A. Randomized Controlled Trials

In the trial by Brodaty et al, elderly patients with a DSM-IV diagnosis of dementia of the Alzheimer's type, vascular dementia, or a combination of the 2 (i.e., mixed dementia) and significant aggressive behaviors were randomized to receive a flexible dose of either placebo or risperidone solution up to a maximum of 2 mg a day, for a period of 12 weeks [16]. These patients were between the ages of 79 and 89 years. Five patients in the risperidone group suffered a stroke and one had a transient ischemic attack (TIA). Five of these patients had vascular or mixed vascular and Alzheimer's type dementia. One patient had Alzheimer's type dementia. Five of the patients had a history of hypertension, 4 of these 6 patients had atrial fibrillation and 1 patient had diabetes mellitus.

In a 42-site, double-blind, placebo-controlled trial of outpatients with Alzheimer's disease and psychosis, aggression, or agitation, patients were randomly assigned to receive olanzapine (mean dose, 5.5 mg per day), quetiapine (mean dose, 56.5 mg per day), risperidone (mean dose, 1.0 mg per day), or placebo for up-to 36 weeks [5]. There were two cerebrovascular events or TIAs in the olanzapine group compared to one each in the quetiapine, risperidone and placebo groups, $P=0.92$.

Table 1. Summary of data from RCTs

Study	Conclusions
Brodaty et al. [16]	More patients in the risperidone group had CVAEs when compared to placebo group during the course of the trial.
Schneider et al. [5]	The risk of CVAEs was no greater in the drug treated group compared to the placebo group.

B. Pooled & meta-analyses and systematic reviews

In a review of the risperidone trials, De Deyn et al. found a total of 29 (3.9%) cerebrovascular adverse events with risperidone treated patients when compared to 7 (1.6%) in placebo treated patients [17]. Of these events, 61.8% were non-specific symptoms such as obtundation, dizziness, or weakness rather than strokes. Serious events including all events with lasting disability, hospitalization, or death as might be expected with stroke were seen in 12 (1.6%) of patients who received risperidone and 3 (0.7%) who received placebo. All patients were aged >85 years. Cerebrovascular adverse events were reported at an average of 30.7 days (range, 3 to 75 days) after beginning treatment with risperidone and 56.7 days (range, 24 to 82 days) for placebo. The incidence of cerebrovascular adverse events did not appear to be dose-dependent (i.e., in 3.7, 3.1, and 5.0% of patients receiving risperidone <0.75, 0.75 to <1.5, and ≥ 1.5 mg/day, respectively). A total of five patients died of a cerebrovascular adverse event, four (0.5%) in the risperidone-treated and one (0.2%) in the placebo-treated group.

In a post hoc analysis of pooled results from 11 randomized controlled trials of risperidone and olanzapine in elderly dementia subjects, Herrmann and Lanctot found an increased incidence of cerebrovascular adverse events when compared to placebo treated patients [18]. A total of 48 out of the 2187 (2.2%) drug treated patients experienced CVAEs when compared to 10 out of the 1190 (0.8%) placebo treated subjects. The combined relative risk was 2.7, 95% confidence interval, (CI), 1.4 to 5.3. Numerically more risperidone treated patients (33 out of 1009, 3.3%) experienced CVAEs when compared to olanzapine treated patients (15 out of 1178, 1.3%). The weighted relative risk was statistically significant for risperidone, 3.2, 95% CI, 1.4 to 7.2, $P=0.0004$, but not for olanzapine, 1.8, 95% CI 0.5 to 6.3, $P=0.36$. A reanalysis of the risperidone trials suggested that some of the increased incidence may have been accounted for by nonspecific events that were not strokes. A larger number of subjects in the risperidone trials were diagnosed with vascular and mixed dementias when compared to the olanzapine trials. This most likely accounted for the increased incidence of cerebrovascular adverse events in the risperidone trials when compared to the olanzapine trials.

In their meta-analysis, Schneider et al. included fifteen trials of 16 contrasts of atypical antipsychotics in the treatment of BPSD [19]. They included trials of aripiprazole (3), olanzapine (5), quetiapine (3) and risperidone (5). There were a total of 3,327 patients in the drug treated group compared to 1,728 in the placebo group. In all there were 63 CVAEs in the drug treated group when compared to 16 events in placebo treated group. There was an increased Odds Ratio (OR) by meta-analysis for CVAEs of 2.13 95% CI, 1.20 to 3.75, $P=0.009$, 1.9% versus 0.9% pooled. There was a significantly increased risk with risperidone $OR=3.43$, 95% CI, 1.60–7.32, $P=0.001$, 3.1% versus 1.0% pooled.

In the meta-analysis by Ballard et al, the investigators found that demented patients treated with risperidone and olanzapine had a higher incidence of cerebrovascular events (including stroke) than placebo treated patients [8]. Risperidone treated patients were significantly more likely to have serious adverse cerebrovascular events, OR, 3.64, 95% CI, 1.72 to 7.69, $P=0.0007$.¹⁵ The investigators concluded that the risk of stroke was similar for olanzapine.

In a review of three placebo-controlled trials of elderly patients with psychosis associated with Alzheimer's disease who were treated with aripiprazole, the investigators found that cerebrovascular adverse reactions (e.g., stroke, transient ischaemic attack), including fatalities occurred in 1.3% of aripiprazole-treated patients compared to 0.6% of placebo-treated patients [20]. Although this difference was not statistically significant, in one fixed-dose trial, there was a significant dose response relationship for cerebrovascular adverse reactions in patients treated with aripiprazole.

Sacchetti et al. completed a systematic review on quantitative reviews on stroke and antipsychotics [21]. The investigators found the incidence of cerebrovascular accidents (CVAs) to be between 2% to 4% in the drug treated group which was statistically significant. The investigators also found that preliminary data indicates that the highest risk of stroke was related to the first weeks of treatment and the rates were higher in older patients, cognitive impairment and vascular illness.

Table 2. Summary of data from pooled analyses, meta-analyses and systematic reviews

Study	Conclusions
De Deyn et al. [17]	Increased risk of CVAEs with risperidone compared to placebo.
Herrmann and Lanctot [18]	Increased risk of CVAEs with risperidone and olanzapine compared to placebo.
Schneider et al. [19]	- The risk of CVAEs in patients treated with the atypical antipsychotics was higher than placebo treated patients. - The risk of CVAEs was 3 times higher in risperidone treated patients compared to placebo treated patients.
Ballard and White [8]	- The risk of CVAEs in the risperidone treated patients is significantly higher than in the placebo treated patients. - Although the data for olanzapine was not made available, the risk of CVAEs appears similar to that of risperidone.
European Agency [20]	- The risk of CVAEs with aripiprazole was not statistically significant. - In one trial (a fixed-dose trial), there was a significant dose response relationship for CVAEs in patients treated with aripiprazole.
Sacchetti et al ²	- The incidence of CVAEs was found to be very low (2-4%). - The risk of CVAEs in the exposed group is about 1.3-2 times the non-exposed groups. - The risk of CVAEs in the typical and the atypical antipsychotic group is similar. - Highest risk of CVAEs is related to the first weeks of treatment. - Risk is higher in those who are older, with cognitive impairment and vascular illness.

C. Other Studies

In a retrospective population-based cohort Herrmann et al. found that the crude stroke rate per 1,000 person-years did not significantly differ among the patients treated with typical antipsychotics (5.7), risperidone (7.8), and olanzapine (5.7) [22]. Relative to typical antipsychotic users, the risk ratio for stroke was 1.1, 95% CI, 0.5 to 2.3 for olanzapine users and 1.4, 95% CI, 0.7 to 2.8 with risperidone users. Relative to olanzapine the users of risperidone were not at significantly increased risk of stroke-related hospital admission, adjusted risk ratio 1.3, 95% CI, 0.8 to 2.2. The investigators concluded that olanzapine and risperidone use was not associated with a statistically significant increased risk of stroke when compared to typical antipsychotic use.

Finkel et al. found that the use of risperidone and other antipsychotics were also not associated with a higher odds ratio (OR) of incident CVAEs when compared to either haloperidol or benzodiazepines [23]. The percentage of patients who had CVAEs in each group were; risperidone (0.86%), olanzapine (0.87%), quetiapine (0.56%), haloperidol (1.19%) and benzodiazepines (1.04%). When risperidone was used as the reference group the

odds ratio were: olanzapine, 1.05, 95% CI, 0.63 to 1.73; quetiapine, 0.66, 95% CI, 0.23 to 1.87; haloperidol, 1.91, 95% CI, 1.02 to 3.60; benzodiazepines, 1.97, 95% CI, 1.30 to 2.98. With benzodiazepines as the reference group, the OR of incident CVAEs for all antipsychotics as a class was 0.49, 95% CI, 0.35 to 0.69. In this study the significant predictors for CVAEs were prior cerebrovascular events and atrial fibrillation. The presence of hypertension was significant in the risperidone versus olanzapine comparison. Age, gender, indicators for atherosclerosis, diabetes, anti-clotting medications and vascular dementia were not significant predictors for CVAEs in this study.

Liperoti et al. in a case-control study on residents of nursing homes in 6 U.S. states, found the odds ratio of being hospitalized for CVAEs to be 0.87, 95% CI, 0.67 to 1.12 for risperidone users, 1.32, 95% CI, 0.83 to 2.11 for olanzapine users, 1.57, 95% CI, 0.65 to 3.82 for users of other atypical agents and 1.24, 95% CI, 0.95-1.63 for conventional antipsychotic users when compared to nonusers of antipsychotics [24]. The investigators also found that users of risperidone and conventional antipsychotics were not at increased risk of being hospitalized for CVAEs regardless of the presence of a previous history of stroke or TIA; adjusted OR, 1.49, 95% CI, 0.93 to 2.38 and 1.23, 95% CI, 0.68 to 2.23 respectively. However, among olanzapine users and users of other atypical agents a history of CVEs increased the risk of being hospitalized for CVEs, adjusted OR, 3.71, 95% CI, 1.55 to 8.84 and 4.63, 95% CI, 1.35 to 32.63 when compared to nonusers without such history.

In a cross sectional study, Formiga et al. included two hundred fourteen women and 106 men with dementia. The mean duration for follow-up was 20 months. 28 One hundred ninety-one patients (60%) had received treatment with neuroleptic drugs: 168 risperidone (53%), 10 haloperidol, six levomepromazine, four olanzapine, two quetiapine, and one thioridazine. The daily dose of risperidone ranged from 0.5 mg to 3 mg, and the mean time since the start of risperidone was 10.1 ± 12.0 months. Six patients experienced a new stroke during follow-up (mean 20 months): three of them in the risperidone group and three in the other group (two patients not taking neuroleptics and one on thioridazine); there were no significant differences ($P = 1.0$). Four patients experienced a TIA during follow-up (mean 18.4 months): one in the risperidone group and three in the other group (two not taking neuroleptics and one taking levomepromazine) ($P = 0.34$). Overall, 10 patients experienced some CVAE during follow-up (mean 19 months): four in the risperidone group and six in the other group (four not taking neuroleptics and one each on thioridazine and levomepromazine) ($P = 0.52$). The risk of strokes or TIAs in patients who had taken neuroleptics was no greater than those who had not taken any neuroleptics, 4 vs 2; $P = 0.90$ and 2 vs 2; $P = 0.90$.

Gill et al. in a population based retrospective cohort study followed 32,710 older adults (≥ 65 years) with dementia who were treated with antipsychotics [26]. Patients were observed until they were either admitted to hospital with ischemic stroke, stopped taking antipsychotics, died, or the study ended. After adjustment for confounding factors, participants receiving atypical antipsychotics showed no significant increase in risk of ischemic stroke compared with those receiving typical antipsychotics, adjusted hazard ratio 1.01, 95% CI, 0.81 to 1.2. The risk of stroke for patients receiving the individual drugs were as follows; risperidone, adjusted hazard ratio (HR) 1.04, 95% CI, 0.82 to 1.31, olanzapine 0.91, 95% CI, 0.62 to 1.32 and quetiapine 0.78, 95% CI, 0.38 to 1.57. This was not significantly different from that of patients receiving typical antipsychotics. The investigators found higher incidence rates of stroke in patients with atrial fibrillation who were prescribed atypical antipsychotics, adjusted HR 1.23, 95% CI, 0.70 to 2.02. Chronic users of atypical

antipsychotics were also not at increased risk of stroke compared with chronic users of typical antipsychotics, adjusted HR 0.89, 95% CI, 0.69 to 1.17.

In a 12 month controlled open-label study of nursing home patients, Moretti et al. included patients who were aged between 71 and 92 [27]. Patients were divided into two groups. One group received olanzapine 2.5 to 7.5 mg a day while the other group received typical neuroleptics (60 patients receiving promazine chloridrate 4%, up to 10 drops three times a day and 113 patients received haloperidol 0.2%, up to 10 drops three times a day). A total of 356 patients were included in this study and their mean age was 76.8 ± 4.01 years. In the two groups, the change from baseline on the Hachinski Ischemic Score were $+1.7 \pm 0.6$ and $+1.6 \pm 0.3$ respectively. The change Matthew's Stroke Scale were -5.4 ± 0.7 and -6.1 ± 0.7 respectively. These changes were not statistically.

Layton et al. compared the incidence rates for events reported as cerebrovascular accident (CVA) and transient ischaemic attack (TIA) during the first 180 days of treatment in patients prescribed atypical antipsychotics for dementia or other indications [28]. This analysis included 7684 risperidone treated patients, 1726 quetiapine treated patients and 8826 olanzapine treated patients. Among the risperidone, quetiapine and olanzapine cohorts, 23 (0.30%), 6 (0.35%) and 10 (0.11%) patients were reported to have had a CVA or TIA. Although age, sex and indication for prescription (dementia or other diagnoses) were identified as important confounding variables, age was being to be found to be the most important variable for developing a CVA or TIA. The crude rate ratios (RRs) for CVA or TIA for risperidone or quetiapine vs. olanzapine indicated an approximate threefold relative difference in rate during the first six months but after adjustment for age, sex and indication, the RRs were non-significant 1.2, 95% CI, 0.5 to 3.0 and 2.1, 95% CI, 0.6 to 7.7, respectively. For risperidone vs. quetiapine, crude and adjusted RRs were not significantly different, 0.84, 95% CI, 0.34 to 2.07, 0.73, 95% CI, 0.27 to 2.00. Of the three drugs, the time to event was shortest for risperidone, 58 days, range 0.5-168 days. The time was also shortest for risperidone and quetiapine users where the indication for use was dementia. The RR of CVA or TIA in patients prescribed risperidone for dementia when compared to other indications was 6.7, 95% CI, 2.4 to 18.9. The adjusted RRs for quetiapine, according to indication, could not be calculated due to missing data. There were no cases of CVA or TIA in the subgroup of demented patients who were prescribed olanzapine and hence the RRs and time to event curves based on indication could not be examined. The investigators concluded that dementia appeared to be an important risk factor for CVA or TIA, RR, 54.52, 95% CI, 19.41 to 153.16 and 16.91, 95% CI, 2.32 to 120.5 for the risperidone and quetiapine groups respectively.

Percudani et al. investigated the relationship between exposure to second-generation antipsychotics (SGAs) and the occurrence of cerebrovascular accidents in the elderly in a database review [29]. The analysis of cerebrovascular events in elderly subjects exposed to each individual SGA when compared to exposure to haloperidol, showed an increased risk only for risperidone, adjusted odds ratio, 1.43, 95% CI, 1.12 to 1.93.

Kolanowski et al. conducted an analysis of the claims data from a large health care insurer in the southeast region of the United States [30]. The investigators included only those cases that had at least one prescription claim for the 3-year period and the observation period was 45 days. In this study, the investigators found that the odds ratio (OR) for stroke was 0.98, 95% CI, 0.64 to 1.52 when the prescription was for typical antipsychotics. The OR was 1.18, 95% CI, 0.63 to 2.24 when the prescription was for atypical antipsychotics. The combined OR was 1.22, 95% CI, 0.55 to 2.68, $P = 0.91$.

In a prospective study of patients aged ≥ 65 years by Barnett et al, patients were observed until they were admitted to a hospital for a cerebrovascular event (CVE), stopped taking or switched antipsychotics, died or until the 18-month observation period ended [31]. After controlling for age, sex, race, marital status, veteran status, comorbid conditions, previous CVE, and prescription drug therapy, the risk of a CVE admission did not differ in those receiving FGA or SGA when compared to those not taking antipsychotics RR 1.20, 95% CI, 0.83 to 1.73. Patients with vascular dementia were found to have increased risk admission for CVE when compared to patients with Alzheimer dementia, RR, 1.14, 95% CI, 1.07 to 1.34. No difference in the risk of CVE admission was found in patients receiving either quetiapine, olanzapine, or risperidone, relative to haloperidol RR, 0.70, 95% CI, 0.30 to 1.65, 0.62, 95% CI, 0.25 to 1.53, 0.49, 95% CI, 0.21 to 1.12. Patients receiving quetiapine, olanzapine or risperidone, relative to haloperidol showed the RR to be 1.04, 95% CI, 0.33-3.91, 1.00, 95% CI, 0.30 to 3.35, 0.80, 95% CI, 0.26 to 2.43 for Alzheimer's dementia and 0.32, 95% CI, 0.07 to 1.46, 0.23, 95% CI, 0.04-1.38, 0.14, 95% CI, 0.02 to 1.68 for vascular dementia. The risk associated with vascular dementia patient relative to Alzheimer dementia patients was significant RR 1.39, 95% CI, 1.04 to 2.11. There was a decreased risk for CVE in vascular dementia patients receiving risperidone relative to those receiving haloperidol RR, 0.13, 95% CI, 0.03 to 0.63. No differences were seen in patients receiving either olanzapine or risperidone, compared to quetiapine RR, 0.84, 95% CI, 0.36 to 2.11, 0.73, 95% CI, 0.41 to 1.76 respectively. In this subgroup analysis, the RR for patients with Alzheimer's disease when compared to vascular dementia was 0.96, 95% CI, 0.39 to 2.32, 0.79, 95% CI, 0.36 to 1.71 respectively. The overall RR for vascular dementia relative to Alzheimer dementia was 1.08, 95% CI, 0.55 to 2.18.

In a study by Wang et al, patients who were 65 years old or older and filled a first recorded (index) prescription for an oral antipsychotic medication between January 1, 1994 to December 31, 2003 and had both parts A and B on all Pennsylvania Pharmaceutical Assistance Contract were included [32]. Atypical antipsychotic drugs aripiprazole, clozapine, olanzapine, quetiapine, risperidone and ziprasidone were included in this review. Other antipsychotics were considered as conventional drugs. By 30 days, adjusted hazards were not higher for conventional than atypical antipsychotics in developing cerebrovascular events, 1.08 95% CI, 0.99 to 1.18. By 60 days, conventional versus atypical antipsychotics was associated with a significantly increased hazard of developing cerebrovascular events, 1.10, 95% CI, 1.02 to 1.19. The hazards of developing cerebrovascular events were also significantly greater for conventional drugs than atypical antipsychotic use at 120 days 1.09, 95% CI, 1.02 to 1.16.

In a retrospective review of a Health Search Database, Sacchetti et al. included all elderly patients (≥ 65 years) who were prescribed an antipsychotic in monotherapy from January 2000 to June 2003 [33]. The comparison group was a cohort of patients who were not exposed to antipsychotics. The main outcome measure was the incidence of first-ever stroke during exposure to an antipsychotic. The crude incidence of stroke in subjects not exposed to antipsychotics was 12.0/1000 person-years, 95% CI, 11.5 to 12.5. The risk was significantly higher in those on butyrophenones, 47.1/1000, 95% CI 22.1 to 88.8, phenothiazines 72.7/1000, 95% CI, 43.3 to 107.7 and in the atypical antipsychotic group 47.4/1000; CI 23.4 to 86.5. Patients on substituted benzamides were also at higher risk, 25.0/1000, 95% CI, 12.0 to 34.1. The mean interval from first prescription to new-onset

stroke was 103.3 days, SD 112.7 in atypicals, 57.5, SD 22.2 in butyrophenones, 21.3, SD 18.8 in phenothiazines and 120.5, SD 119.6 in substituted benzamides. The risk of stroke was again higher for the group on phenothiazines, 5.79 times, 95% CI, 3.07 to 10.9, butyrophenones, 3.55, 95% CI, 1.56 to 8.07 and atypical antipsychotics, 2.46, 95% CI, 1.07 to 5.65 when compared with unexposed subjects. The group on substituted benzamides also had significantly higher risk, 2.2, 95% CI, 0.98 to 4.90. Phenothiazines also had a significantly higher risk, 2.34, 95% CI, 1.01 to 5.41 for stroke when compared with atypical antipsychotics. Older age, male sex, a Chronic Disease Score greater than 5, a diagnosis of Parkinson disease and the use of anticoagulants increased the risk of strokes. The diagnosis of dementia only had a weak effect on stroke risk and affective disorders were not associated with a risk for stroke.

In an electronic primary care records review by Douglas and Smeeth, the investigators identified 6790 eligible patients in the database with at least one prescription for an antipsychotic drug and a recorded incident stroke between January 1988 and the end of 2002 [34]. Of these 64% were women. The most commonly used typical antipsychotics were phenothiazines (5153 patients), and the most common atypical antipsychotic drug was risperidone (729 patients). The median age at first exposure to any antipsychotic drug was 80 years, while median age at the time of first recorded stroke was 81 years. The use of any antipsychotic drug was associated with a rate ratio for stroke of 1.73, 95% CI, 1.60 to 1.87. The rate ratio was 1.69, 95% CI, 1.55-1.84 for typical antipsychotics and 2.32, 95% CI, 1.73-3.10 for atypical antipsychotics. In patients receiving any antipsychotic drug, the rate ratios were 3.50, 95% CI, 2.97 to 4.12 for those with dementia and 1.41, 95% CI, 1.29 to 1.55 for those without dementia. In patients with dementia prescribed typical antipsychotics, the rate ratio for stroke was 3.26, 95% CI, 2.73 to 3.89. In patients with dementia treated with atypical antipsychotics, the rate ratio was 5.86, 95% CI, 3.01 to 11.38. Patients without dementia prescribed typical antipsychotics had a rate ratio of 1.40, 95% CI, 1.26 to 1.54 compared to 1.90, 95% CI, 1.36 to 2.65 to those prescribed atypical antipsychotics.

In a case-controlled analysis by Kleijer et al, a cohort of 26,157 community-dwelling patients (mean age 76 ± 9.7 years) with at least one antipsychotic prescription were included in the analysis [35]. Data from community pharmacies and hospital discharge records were utilized. Five hundred and eighteen cases of hospital admission for CVAE were identified. After adjustment baseline risk, current use of antipsychotics was associated with an increased risk of CVAE, adjusted OR, 1.6, 95% CI, 1.3 to 2.0. Patients who had a recent prescription (8 to 30 days ago) had a higher risk of CVAE compared with non-users, adjusted OR, 2.0, 95% CI, 1.3 to 3.3. Past use (>30 days ago) was not associated with an increased risk of CVAE, adjusted OR, 1.2, 95% CI, 0.9 to 1.6. There was a tenfold increase in risk of CVAE in the first week of use compared with controls, adjusted OR 9.9, 95% CI, 5.7 to 17.2. This risk decreased over time and was back on baseline level after 3 months of treatment, adjusted OR, 1.0, 95% CI, 0.7–1.3. There were no differences in risk between ischemic and haemorrhagic strokes. Exclusion of transient ischemic attacks (TIA's) in the analysis did not change the results. Cumulative exposure did not increase the risk for CVAE, ORs, 95% CI, 0.9 to 1.1. Users of conventional antipsychotics had an increased risk compared with users of atypical antipsychotics, OR 1.6, 95% CI 1.0 to 2.5; current users: OR 2.6, CI 1.3 to 5.0.

Table 3. Summary of data from other studies

Study	Conclusions
Herrmann et al. [22]	Olanzapine and risperidone use was not associated with a statistically significant increased risk of stroke compared with typical antipsychotic use.
Finkle et al. [23]	- Risperidone has similar risk of CVAEs compared to olanzapine, quetiapine and haloperidol. - The risk of CVAEs with antipsychotics is no greater than with benzodiazepines
Liperoti et al. [24]	- Users of risperidone and conventional antipsychotics had no increased risk of being hospitalized for CVAEs regardless of the presence of a previous history of stroke/TIA. - Olanzapine users and users of other atypical agents presenting with a history of CVEs were more likely to be hospitalized for CVEs compared to nonusers without such history
Formiga et al. [25]	No significant differences in CVAEs when comparing risperidone to haloperidol, levopromazine, olanzapine, quetiapine and thioridazine.
Gill et al. [26]	- No difference in the risk of ischemic CVAEs comparing atypical to typical antipsychotics. - The risk of ischemic CVAEs is similar for risperidone, olanzapine and quetiapine. - Atrial fibrillation may be a risk factor for ischemic CVAEs in patients treated with atypical antipsychotics.
Moretti et al. [27]	There is no statistically significant increase in risk of CVAEs with olanzapine compared to typical antipsychotics; promazine and haloperidol.
Layton et al. [28]	- The risk of CVAEs is similar for risperidone and quetiapine when compared to olanzapine. - Dementia appeared to be an important risk factor for CVAEs when treated with risperidone and quetiapine.
Percudani et al. [29]	The risk of CVAEs is similar in second generation antipsychotics compared to first generation antipsychotics except for risperidone where the risk may be higher.
Kolanowski et al. [30]	The risk of CVAEs was no greater in the typical or atypical antipsychotic group compared to placebo group.
Barnett et al. [31]	- There was no increase in the risk for CVAEs with FGA or SGA in the analysis stratified by type of dementia. - Overall, CVAE risk did not differ whether patients were receiving a FGA, SGA, or no antipsychotic therapy. - Patients with vascular dementia had an increased risk in hospitalization for CVAEs.
Wang et al. [32]	The risk of CVAEs is higher with conventional antipsychotics compared to atypical antipsychotics by 60 days and continues to 120 days.
Sachetti et al. [33]	- Phenothiazines had a significantly higher risk for CVAEs when compared with atypical antipsychotics. - Higher age, male sex, a Chronic Disease Score higher than 5, a diagnosis of Parkinson disease and the use of anti-coagulants increased the risk of CVAEs. - Dementia, <i>per se</i>, had only a weak effect on CVAEs and affective disorders were not associated to the risk.

Study	Conclusions
Douglas and Smeeth [34]	• All antipsychotics are associated with an increased risk of CVAEs. • The risk of CVAEs might be higher in patients receiving atypical antipsychotics than those receiving typical antipsychotics. • Patients with dementia appear to be at higher risk of CVAEs when treated with these medications.
Kleijer et al. [35]	• Current use of antipsychotics was associated with an increased risk of CVAE. • Recent prescription (8 to 30 days ago) had a higher risk of CVAE compared with non-users. • Past use (>30 days ago) was not associated with an increased risk of CVAE. • Cumulative exposure did not increase the risk for CVAE. • Users of conventional antipsychotics had an increased risk compared with users of atypical antipsychotics.
Chan et al. [36]	• The risk of developing CVAEs did not differ in typical or atypical antipsychotic user groups compared with non-user group. • Subgroup analyses of individual antipsychotic did not show a significant increase in risk of CVAEs.
Pratt, et al. [37]	• The incidence rate ratio (IRRs) for stroke was significantly raised in the first week following the initiation of a typical antipsychotic medication and returned to baseline level after 8 weeks of exposure. • No significant increase in the risk of stroke was found in any post-exposure period in patients taking atypical antipsychotics. • The risk of hospitalization for stroke was more than seven times greater in the week prior to initiating a typical antipsychotic but not an atypical antipsychotic medication. • Both groups had significantly increased risks of hospitalization for stroke up to 20 weeks prior to initiating therapy.

In a retrospective cohort study Chan et al. included patients aged 65 or above, who were diagnosed with Alzheimer's disease, vascular or mixed dementia and were admitted to an inpatient unit between 1st January 2000 to 30th June 2007 [36]. The patients were divided into three groups; typical antipsychotics, atypical antipsychotics and placebo groups. A total of 1089 patients were included in this study. Incidence rate of CVAE for the three groups were 44.6/1000, 32.7/1000 and 49.6/1000 person years respectively. The adjusted hazard ratio for the typical and atypical antipsychotic user groups were 0.964, 95% CI, 0.584 to 1.591 and 1.036, 95% CI, 0.350 to 3.066 when compared to placebo group. Subgroup analyses of individual antipsychotics did not show a significant increase in risk of CVAEs.

In a self-controlled case series using the Australian Government Department of Veterans' Affairs administrative claims dataset, Pratt et al. included 10638 patients aged ≥ 65 years with at least one hospitalization for stroke identified during the 4-year period from 1 January 2003 to 31 December 2006 [37]. The incidence rate ratio (IRRs) for stroke, after adjustment for age and calendar year, was significantly raised in the first week following the initiation of a typical antipsychotic medication, IRR, 2.25; 95% CI 1.32 to 3.83 and then returned to baseline levels after 8 weeks of exposure, IRR 0.82, 95%, 0.61 to 1.11. No significant increase in the risk of stroke was found in any post-exposure period in patients taking atypical antipsychotics, IRR, 1.46, 0.83 to 2.56. The risk of hospitalization for stroke was more than seven times greater in the week prior to initiating a typical antipsychotic compared with other

unexposed periods, IRR, 7.22, 95% CI, 5.30 to 9.84. This increased risk of hospitalization was not found in the week prior to initiating an atypical antipsychotic, IRR, 1.24, 95% CI, 0.68 to 2.28. Both groups had significantly increased risks of hospitalization for stroke up to 20 weeks prior to initiating therapy with antipsychotics; typical antipsychotics, IRR, 1.76, 95% CI, 1.25 to 2.49 and atypical antipsychotics, IRR, 1.39, 95% CI, 0.97 to 1.99.

RISK OF DEATH

A. Randomized placebo controlled trials

Brodaty et al. in their study found that 10 patients; 6 (3.6%) in the risperidone group and 4 (2.4%) in the placebo group died during the course of the trial [16]. The most common causes of death was pneumonia; 3 in the risperidone group and 1 in the placebo group and stroke, 2 in the risperidone group.

Schneider et al. found that in their double-blind, placebo-controlled trial of 421 outpatients with Alzheimer's disease and psychosis, aggression, or agitation there was 1 death in the olanzapine and risperidone groups whereas there were 3 deaths each in the quetiapine and the placebo groups [5]. The results were not statistically significant, $P = 0.58$.

In the study conducted by Ballard et al. in AD patients who resided in longterm care facilities in the United Kingdom were enrolled into a randomized, placebo-controlled, parallel, two-group treatment discontinuation trial [38]. Participants were randomly assigned to continue with their antipsychotic treatment; thioridazine, chlorpromazine, haloperidol, trifluoperazine, or risperidone for 12 months or to switch their medication to an oral placebo. The primary outcome was mortality at 12 months. Additional follow-up telephone assessment was done to establish whether each participant was still alive 24 months after the enrollment of the last participant, range 24 to 54 months. Causes of death were obtained from death certificates. Analysis was by intention to treat (ITT) and modified intention to treat (mITT). During the 12-month randomized, double-blind phase of the trial, comparisons between the patients who continued antipsychotic treatment and the placebo group during the showed that the cumulative probabilities of survival were 89.7%, 95% CI, 71.3% to 96.5% versus 97.1%, 95% CI, 80.9% to 99.6% in the patients who continued to take their allocated treatment for 12 months, 70.3%, 95% CI, 57.5% to 79.9% versus 76.6%, 95% CI, 64.2% to 85.2% in the patients who started their allocated treatment (mITT population), and 74.7%, 95% CI, 63.9% to 82.7% versus 79.3%, 95% CI, 68.8% to 86.6% in the ITT population. The Kaplan–Meier estimates show that during the extended follow up phase, the patients who continued antipsychotic treatment had significantly higher mortality compared with those who took placebo mITT population log rank $P = 0.03$, HR 0.58, 95% CI, 0.35 to 0.95; ITT population log rank $P = 0.02$, HR 0.58, 95% CI, 0.36–0.92. The difference in mortality was more pronounced after the first year. In the mITT population, the cumulative survival was 46% versus 71% between the continued treatment and placebo groups at 24 months, 30% versus 59% at 36 months, and 26% versus 53% at 42 months. The numerical differences at months 24, 36, and 42 were similar in the ITT population and the patients who took their medication for the first 12 months.

Table 1. Summary of data from RCTs

Author	Conclusions
Brodaty et al. [16]	More patients in the risperidone group died when compared to the placebo group during the trial.
Schneider et al. [5]	The risk of death in the drug treated group was similar to the placebo group.
Ballard et al. [38]	- The probability of survival in the antipsychotic group was less than the placebo group during the first 12 months. - The probability of survival decreases with continued treatment with drugs.

B. Meta-analysis and systematic review

In a meta-analysis by Schneider et al, the investigators evaluated 15 trials of 10 to 12 weeks duration which included 16 contrasts of atypical antipsychotic drugs with placebo met criteria; aripiprazole [n = 3], olanzapine [n = 5], quetiapine [n = 3], risperidone [n = 5] [39]. Death occurred more often among patients randomized to drugs 118 [3.5%] versus placebo 40 [2.3%]. The odds ratio (OR) by meta-analysis was 1.54, 95% CI, 1.06 to 2.23, P= 0.02; and risk difference was 0.01, 95% CI, 0.004 to 0.02, P= 0.01. Sensitivity analyses did not show evidence for differential risks for individual drugs, severity, sample selection, or diagnosis.

In the European Medicines Agency review discussed earlier, the rate of death in aripiprazole-treated patients was 3.5% compared to 1.7% in the placebo group [20]. Most of the deaths appeared to be either cardiovascular (e.g., heart failure, sudden death) or infectious (e.g., pneumonia) in nature.

Table 2. Summary of data from pooled analyses, meta-analyses and systematic reviews

Author	Conclusions
Schneider et al. [40]	Death occurred more often in the drug treated group compared to placebo group.
European Agency [20]	The rate of death is higher in the aripiprazole group compared to the placebo group.

C. Population based studies, retrospective analyses and other studies

In an observational study by Nasrallah et al, the investigators tracked the mortality over a 2-year period in patients aged ≥ 65 years who were receiving haloperidol (N=299) or the atypical antipsychotics risperidone or olanzapine (N= 1,254) [41]. Sixty-four patients in the haloperidol group (21.4%) and 61 patients in the atypical group (4.75%) died during the 2-year study period, P <0.00001.

In a prospective study by Raivio et al, elderly institutionalized patients with dementia were followed for up for two years [42]. Of these patients, 48.4% were prescribed antipsychotic medication: 37.4% received conventional neuroleptics and 11.0% received atypical antipsychotics. Of the users of atypical antipsychotics (risperidone, olanzapine),

32.1% died within 2 years. The respective figures for users of conventional neuroleptics were 45.3%, and for the nonusers, 49.6%. When comparing the users of any antipsychotic to the nonusers, the mortality was higher among the nonusers (72%) than among the users (58%), $P = 0.019$. Mortality was at first year among users 17% and among nonusers 27%, $P = 0.064$ and at two years 42% and 50%, respectively, $P = 0.24$. High number of medications and the use of physical restraints predicted higher mortality at two years; hazard ratio 1.12, 95% CI, 1.05 to 1.20, $P < 0.001$, 1.72, 95% CI, 1.04 to 2.83, $P = 0.03$ respectively. The use of atypical antipsychotic agents appeared to decrease the mortality risk and the effect of conventional antipsychotics was non-significant; hazard ratio 0.49, 95% CI, 0.24 to 0.99, $P = 0.047$, 0.68, 95% CI, 0.46 to 1.03, $P = 0.069$.

Barnett et al. evaluated VA patients admitted for pneumonia in fiscal year (FY) 2003 for exposure to typical and atypical antipsychotics and other neuropsychiatric drugs based on a prescription within 120 days preceding admission [43]. The odds of in-hospital mortality for patients admitted with pneumonia was higher for recent exposure to typical antipsychotics OR = 1.51, 95% CI, 1.04 to 2.19, $P = 0.03$ when compared to patients not receiving psychotropic medications. There was no significant difference in in-hospital mortality in patients exposed to atypical antipsychotics OR 1.20, 95% CI, 0.96 to 1.50, $P = 0.10$, tricyclic antidepressants OR 1.20, 95% CI, 0.44 to 1.55, $P = 0.15$, other antidepressants OR 1.07, 95% CI, 0.93 to 1.23, $P = 0.37$ or mood stabilizers OR 0.91, 95% CI, 0.73 to 1.14, $P = 0.41$.

In the study by Suh and Shah, the investigators measured the rate of mortality in patients with dementia, Alzheimer's disease (AD) and vascular/mixed dementia who were antipsychotics [44]. The 1-year mortality rate in dementia was 23.8%. The mortality rate in those who had not received antipsychotics (26.8%) was higher than that in those who had received antipsychotics (20.6%), unadjusted RR 1.168, 95% CI, 1.100 to 1.239. After adjusting for age and severity of dementia, the 1-year mortality remained higher in those who had not received antipsychotics adjusted model I: RR 1.202, 95% CI 1.107 to 1.324. When cerebrovascular disease, cardiovascular disease, hypertension, diabetes mellitus were added to the adjusted model I along with age and severity of dementia, the adjusted model II, RR was 1.211, 95% CI 1.107 to 1.324. When the total MMSE score was added to the adjusted model II, the relative risk was 1.244, 95% CI, 1.122 to 1.379 (adjusted model III). When both total scores of the Mini-mental State Examination (MMSE) and the Behavioral Pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD) were added, the relative risk of mortality was 1.277, 95% CI 1.134–1.437 (adjusted model IV). Comparing those who had received both risperidone and haloperidol similar patterns were observed. The relative risks for all dementia, AD and vascular/mixed dementia in adjusted model IV, adjusted for age, severity of dementia, medical comorbidities, cognitive impairment measured by MMSE and BPSD measured by BEHAVE-AD were 1.225, 95% CI, 1.101 to 1.364, 1.226, 95% CI, 1.063–1.414 and 1.154, 95% CI, 0.959–1.389 respectively.

In an observational cohort study by Nonino et al, the investigators included a cohort of patients ≥ 65 years of age with BPSD who were treated with atypical antipsychotics and a cohort of demented patients who were not prescribed any atypical antipsychotics [45]. The measured outcomes were death by any cause and death by cerebrovascular accident at the end of the study with a median follow-up of one year. At one year, lower mortality rate was noted in the cohort not treated with antipsychotics, overall mortality rate 0.52 vs. 0.55/1000 years/person, rate ratio 0.95, 95% CI, 0.71 to 1.25; relative risk reduction 0.047, 95% CI, – 0.251 to 0.286. Mortality was higher among men, 0.74 vs. 0.46/1000 years/person, rate ratio

1.61, 95% CI, 1.33 to 1.94 and among patients with older age at entry, 0.33, 0.37, 0.61, 0.87 for ages 65 to 77, 78 to 82, 83 to 87 and 88 to 105 years respectively. Older age at entry, male gender, severe dementia and functional impairment were associated with a higher risk of death; hazard ratio 1.06, 95% CI, 1.04 to 1.08, $P < 0.001$, 2.20, 1.76 to 2.75, $P < 0.001$, 1.67, 95% CI, 1.32-2.11, $P < 0.001$, 1.81, 1.41-2.33, $P < 0.001$ respectively.

Gill et al. completed a population-based, retrospective cohort study in older adults with dementia [46]. The investigators ascertained the risk for death at 30, 60, 120, and 180 days after the initial dispensing of an antipsychotic medication. Two pairwise comparisons were made: atypical versus no antipsychotic use and conventional versus atypical antipsychotic use. New use of atypical antipsychotics was associated with a statistically significant increase in the risk for death at 30 days when compared with nonuse in both the community-dwelling cohort, adjusted hazard ratio, 1.31, 95% CI, 1.02 to 1.70; absolute risk difference, 0.2 percentage point and the long-term care cohort adjusted hazard ratio 1.55, 95% CI, 1.15 to 2.07; absolute risk difference, 1.2 percentage points. Excess risk seemed to persist to 180 days, although unequal rates of censoring over time may have affected these results. Conventional antipsychotics were associated with an even greater risk for death at 30 days. The adjusted hazard ratio was 1.55, 95% CI, 1.19 to 2.02 for the community-dwelling cohort and 1.26, 95% CI, 1.04 to 1.53 for the long-term care cohort adjusted risk difference for both groups, 1.1 percentage points. The risk persisted to 180 days, adjusted hazard ratio 1.23, 95% CI, 1.00 to 1.50; absolute risk difference, 2.6 percentage points and 1.27, 95% CI, 1.09 to 1.48; absolute risk difference, 2.2 percentage points, respectively.

In a population-based cohort study, Schneeweiss et al. assessed the short-term mortality in those older subjects who were prescribed conventional and atypical antipsychotic medications [47]. The investigators compared the 180-day all-cause mortality between residents taking conventional antipsychotic medications and those taking atypical antipsychotic medications. Within the first 180 days of use, 1822 patients (14.1%) in the conventional drug group died, compared with 2337 (9.6%) in the atypical drug group, mortality ratio 1.47, 95% CI, 1.39 to 1.56, adjusted mortality ratio, 1.32, 95% CI, 1.23 to 1.42. When compared to risperidone, haloperidol was associated with the greatest increase in mortality; mortality ratio 2.14, 95% CI, 1.86 to 2.45. Loxapine had the lowest mortality ratio, 1.29, 95% CI, 1.19 to 1.40. The greatest increase in mortality occurred among people taking higher than median doses of conventional antipsychotic medications, mortality ratio 1.67, 95% CI, 1.50 to 1.86 and during the first 40 days after the start of treatment, mortality ratio, 1.60, 95% CI, 1.42 to 1.80.

Liperoti et al. in a retrospective cohort study compared the risk of death associated with atypical and conventional antipsychotics in a population of nursing home residents with dementia [48]. The outcome measure was all-cause mortality at 6-months of follow-up. There were 1,907 deaths during the 6-month follow-up time; the rate of death was 44.6 per 100 person-years. The median follow-up time was 180 days for users of both atypical and conventional antipsychotics. Survival curves for users of atypical antipsychotics and users of conventional agents differed at the Mantel-Haenszel test ($P < 0.001$). The deaths started early at 8 to 10 days but were distributed throughout the entire follow-up time. The rate of death was increased for users of conventional antipsychotics, adjusted hazard ratio (HR), 1.26, 95% CI, 1.13 to 1.42. Relative to risperidone, a higher rate of death was documented for haloperidol HR, 1.31, 95% CI, 1.13 to 1.53, phenothiazines HR, 1.17, 95% CI, 1.00 to 1.38 and other conventional medications HR, 1.32, 95% CI, 0.99 to 1.80. No atypical

antipsychotic was associated with a differential risk relative to risperidone HR, clozapine 0.94, 95% CI, 0.40 to 1.79, olanzapine 0.95, 95% CI, 0.80 to 1.12, quetiapine 1.05, 95% CI, 0.80 to 1.39. The excess mortality associated with conventional antipsychotics was found only among those patients with dementia other than Alzheimer's disease HR, 1.31, 95% CI, 1.14 to 1.50.

Rossom et al. in a retrospective study compared four cohorts of exposed patients; haloperidol (n=2,217), olanzapine (n=3,384), quetiapine (n=4,277) and risperidone (n=8,249) and their risk of death. These cohorts consisted mainly of men aged ≥ 65 and older with a diagnosis of dementia [49]. Those subjects who received an antipsychotic medication were compared with randomly selected controls who did not receive antipsychotics. Cohorts who were exposed to haloperidol, olanzapine, quetiapine or risperidone had more comorbidities than their control cohorts. During the first 30 days, there was a significant increase in mortality in subgroups prescribed a daily dose of haloperidol greater than 1 mg, adjusted hazard ratio (HR) 3.2, 95% CI, 2.2 to 4.5, $P < 0.001$, olanzapine greater than 2.5 mg, HR 1.5, 95% CI, 1.1 to 2.0, $P = 0.01$, or risperidone greater than 1 mg, HR 1.6, 95% CI, 1.1 to 2.2, $P = 0.01$. There was no increase in mortality when the daily dose of quetiapine greater than 50 mg, HR 1.2, 95% CI, 0.7 to 1.8, $P = 0.50$ or less 50 mg, HR 0.7, 95% CI, 0.5 to 1.0, $P = 0.03$. After excluding inpatients and subjects discharged within 30 days and adjusting for any differences in baseline covariates, including time since previous discharge, prescriptions for haloperidol or olanzapine were still associated with greater risk of mortality during the initial 30-days of exposure, adjusted HR 2.3, 95% CI, 1.6 to 2.9, $P < 0.001$ and 1.4, 95% CI, 1.9, $P = 0.03$ respectively. For risperidone the adjusted HR was 1.2, 95% CI, 0.95 to 1.4, $P = 0.13$. Quetiapine was associated with significantly lower risk of mortality, adjusted HR 0.7, 95% CI, 0.5 to 0.97, $P = 0.03$. No antipsychotic was associated with greater mortality after the first 30 days.

In a retrospective population cohort study patients who were aged 60 years or older and newly prescribed antipsychotic drugs were included in the study [50]. They were categorized according to typical (n=156) or atypical (n=806) drug exposure. The mortality risks of users of typical or atypical antipsychotics compared to nonusers were evaluated with survival analysis, considering exposure to antipsychotic drugs as a time-dependent variable. At the end of two years, adjusted death rate was 3.7, 95% CI, 2.6 to 5.1, in those prescribed a typical antipsychotic drug with respect to those who were not prescribed any drug. The adjusted death rate for atypical antipsychotic drug was 2.5, 95% CI, 2.0 to 3.0. The adjusted death rate when comparing typical to atypical drugs were 1.5, 95% CI, 1.1 to 2.1 indicating a 50% increased risk of death. The risk was also higher in men, adjusted death rate, 1.5, 95% CI, 1.2 to 1.8. Death was also higher in those patients with comorbid cancer, adjusted death rate, 4.0, 95% CI, 2.8 to 5.8 and cardiovascular and cerebrovascular disease, adjusted death rate of 1.6, 95% CI, 1.2 to 2.1. Cancer and dementia were the most common causes of death in the typical antipsychotic cohort whereas dementia, respiratory infection, cancer, stroke and non-ischemic heart disease were the most common causes of death in the atypical antipsychotic group.

In a retrospective cohorts study Huybrechts et al, evaluated the risk of death in patients who were ≥ 65 years and had initiated treatment with psychotropics after admission to a nursing home in British Columbia between 1996 and 2006 [51]. Atypical antipsychotics were initiated in 1942 patients, conventional antipsychotics in 1902, antidepressants in 2169 and benzodiazepines in 4887 patients. Compared to users of atypical antipsychotics, users of

conventional antipsychotics and antidepressants had an increased risk of death; rate ratio [RR] 1.47, 95% CI, 1.14 to 1.91, 1.20, 95% CI 0.96 to 1.50 respectively. Users of benzodiazepines had a higher risk of death; RR 1.28, 95% CI 1.04 to 1.58 when compared with users of atypical antipsychotics. Cardiac arrest, ventricular arrhythmia and venous thromboembolism were uncommon events, with rates well below one per 100 person-years in all groups.

In a retrospective cohort study by Kales et al, the investigators compared the 180-day mortality rates in dementia patients aged ≥ 65 years who began outpatient treatment with an antipsychotic medications; risperidone, olanzapine, quetiapine, or haloperidol or valproic acid and its derivatives [52]. In adjusted intent-to-treat analyses, haloperidol was associated with the highest mortality rate (relative risk (RR), 1.54, 95% CI, 1.38 to 1.73) followed by risperidone (used as a reference), olanzapine (RR, 0.99, 95% CI, 0.89 to 1.10), valproic acid and its derivatives (RR, 0.91, 95% CI, 0.78 to 1.06), and quetiapine (RR, 0.73, 95% CI, 0.67 to 0.80). The mortality risk with haloperidol was highest in the first 30 days but decreased significantly and sharply thereafter. Among the other agents, mortality risk differences were most significant in the first 120 days and declined in the subsequent 60 days during follow-up.

In a population based cohort study Huybrechts et al. assessed the risks of mortality associated with use of individual antipsychotic drugs in elderly residents in nursing homes [53]. The investigators used the Cox proportional hazards models to compare the 180 day risks of all cause and cause specific mortality by individual drug with propensity score adjustment to control for potential confounders. The investigators found that compared with risperidone, users of haloperidol had an increased risk of mortality, hazard ratio (HR), 2.07, 95% CI, 1.89 to 2.26 and patients treated with quetiapine had a reduced risk, HR, 0.81, 95% CI, 0.75 to 0.88. The effect of haloperidol was strongest during the first 40 days of treatment, HR, 2.34, 95% CI, 2.11 to 2.60 and reduced to 1.32 (1.02 to 1.71) and 1.46 (1.07 to 2.00) after 40-79 and 80-180 days of treatment respectively. The corresponding rate ratios for quetiapine were 0.74 (0.66 to 0.82), 0.87 (0.75 to 1.01), and 0.91 (0.79 to 1.05). The investigators did not find evidence that the effect measure was modified by the presence of a recorded diagnosis of dementia or behavioral disturbances. Although no difference in overall mortality was observed for olanzapine, findings suggest a possibly lower risk of death from cerebrovascular diseases, HR, 0.88, 95% CI, 0.73 to 1.07. Given the relatively small number of patients treated with aripiprazole and ziprasidone the associations for cause specific mortality were imprecisely estimated. The investigators found a dose-response relation for all antipsychotic drugs except quetiapine. The dose effects were most pronounced for haloperidol, HR, 1.84 for high and 1.40 for medium dose, both compared with low dose and for risperidone (1.35 for high and 1.19 for medium dose). Almost half (49%) of deaths were recorded as caused by circulatory disorders, 10% by cerebrovascular diseases, and 15% by respiratory disorders.

Table 3. Summary of data from other studies

Author	Conclusions
Nasrallah et al. [41]	The risk of death was greater in the haloperidol treated group when compared to the risperidone and the olanzapine treated group.
Raivio et al. [42]	Users of antipsychotics had higher mortality when compared to nonusers.
Barnett et al. [43]	- The odds of in-hospital mortality for patients admitted with pneumonia was higher in those with a recent exposure to typical antipsychotics when compared to nonusers. - Patients exposed to atypical antipsychotics, tricyclic antidepressants, other antidepressants or mood stabilizers had no significant difference in in-hospital mortality.
Suh and Shah [44]	The rate of mortality was higher in the group not receiving any antipsychotic medication.
Nonino et al [45]	There was no increase in mortality in patients treated with atypical antipsychotics.
Gill et al. [46]	- Atypical antipsychotic use is associated with an increased risk for death compared with nonusers. - The risk for death may be greater with conventional antipsychotics than with atypical antipsychotics. - The risk of death is evident from 30 days to 180 days.
Schneeweiss et al. [47]	- The risk of death with conventional antipsychotic medications is comparable to and possibly greater than the risk of death associated with atypical antipsychotic medications. - The risk with typical antipsychotics is greater in doses above the median doses.
Leperoti et al. [48]	- Those receiving conventional agents, especially haloperidol, are at greater risk of death than those on atypical antipsychotics. - The excess mortality associated with the use of conventional antipsychotics is apparent within the initial 8 to 10 days of treatment and persists over 6 months. - The effect of conventional antipsychotics on increasing the risk of death relative to atypical medications seems to be clustered in residents with dementia other than Alzheimer's disease.
Rossum et al. [49]	Commonly prescribed doses of haloperidol, olanzapine, and risperidone, but not quetiapine, were associated with a short-term increase in mortality.
Musicco et al. [50]	- Adjusted death rate with typical antipsychotic drugs was higher than those who were not prescribed any drug or to those prescribed atypical antipsychotic drugs. - Adjusted death was higher in those patients with comorbid cancer and cardiovascular and cerebrovascular disease.
Huybrechts et al. [51]	Compared to users of atypical antipsychotics, users of conventional antipsychotics, antidepressants and benzodiazepines had a higher risk of death.

Author	Conclusions
Kales et al. [52]	• Haloperidol was associated with the highest mortality rate followed by risperidone, olanzapine, valproic acid and its derivatives and quetiapine. • The mortality risk with haloperidol was highest in the first 30 days but decreased significantly thereafter. • Among the other agents, mortality risk differences were most significant in the first 120 days and declined in the subsequent 60 days.
Huybrechts et al. [53]	• Compared with risperidone, users of haloperidol had an increased risk of mortality and patients treated with quetiapine had a reduced risk. • The effect of haloperidol was strongest during the first 40 days of treatment. • A dose-response relation was found for all antipsychotic drugs except quetiapine. • The effect was not modified by the presence of a diagnosis of dementia or behavioral disturbances.

Summary

Available data indicates that the risk of CVAEs is higher in elderly demented patients who are treated with antipsychotic medications. Although preliminary, existing data indicates that the risk is similar in both atypical and typical antipsychotics. No one particular drug has been found to be worse than the other in terms of CVAEs risks when used to treat elderly demented patients. A higher than median dose of a drug, older age, a diagnosis of dementia especially vascular dementia and comorbid atrial fibrillation have all been noted to be risk factors for CVAEs. The risk appears to be highest in the first week of treatment and remains elevated for about 20 months. Potential mechanisms by which antipsychotics can cause CVAEs include the development of orthostatic hypotension and tachycardia, most likely as a result of antagonism at alpha-adrenergic receptors [18] [54]. In individuals with cerebrovascular insufficiency CVAE can occur due to hypotension aggravating an already compromised cerebral perfusion, decreased cerebral perfusion due to rate-induced decrease in diastolic filling and cardiac output or tachycardia dislodging a thrombus in the patient with cardiac arrhythmia. Other mechanisms include hyperprolactinemia accelerating atherosclerosis and increasing the risk of CVAEs, sedation and resulting dehydration causing hemoconcentration and development of a thrombus. A venous thrombus which can embolize to the arterial circulation can also cause CVAEs especially in patients with cardiac arrhythmias [18] [54].

Current data indicates that risk of death with antipsychotics; atypicals and typicals is greater than when compared to the placebo treated group or the group that did not use these medications. Existing data for atypical versus typical antipsychotics indicate that the risk of death appears similar in both groups. No one drug appears safer than the other in terms of the risk of death. Older age, male gender, severe dementia and functional impairment are associated with a higher risk of death. The risk is highest in the first 30 days of prescription and appears to last for up-to 2 years. The mechanisms by which antipsychotic medications increase the risk of death is currently unclear. Most deaths are thought to be due to

cardiovascular events (mostly cardiac failure and arrhythmias) and infections (pneumonia) [48]. Antipsychotics are known to cause QTc prolongation with the highest estimated risk for thioridazine [47]. Increased risk of ischemic cerebrovascular events has been also linked to the use of atypical antipsychotics in patients with dementia [18] [27]. The comorbidities in these demented patients along with the use of these medications only add to this risk [18] [54].

CONCLUSION

Current evidence indicates that the use of antipsychotics increases the risk of CVAEs and deaths in elderly demented patients. Available data indicates that the risks are similar for both atypical and typical antipsychotics. The risk appears higher when used in older patients, in those patients with vascular dementia and atrial fibrillation and when higher than median doses of medications are used. The risk for CVAEs appears to be highest in the first week of treatment and tends to remain high for about 20 months. The risk of death is highest in the first 30 days of treatment and remains high for about 2 years. When using antipsychotics to treat BPSD, these risks should be considered in the context of the patient's age, diagnosis and comorbid conditions. Careful use of these medications with pragmatic evaluation of their risks and benefits will aid in the reduction of potential side-effects like CVAEs and death thereby preventing excessive morbidity and mortality.

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Chapter 53

ULTRASOUND IN THE MANAGEMENT OF ISCHEMIC STROKE

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ABSTRACT

Ultrasound is increasingly being used in the diagnosis, management and treatment of ischemic stroke. Linear array probes with frequencies of 5 to 10 MHz are used for extracranial studies, and sector or small hand-held 2 MHz probes for transcranial studies. Ultrasound is non-invasive, safe, painless, portable, quick, inexpensive, repeatable and accurate, but it requires adequate training; accreditation for physicians and laboratories are available. The extracranial arteries imaged include the common, internal and external carotid, vertebral, subclavian arteries bilaterally, and the brachiocephalic trunk. For transcranial studies, the ophthalmic artery, carotid siphon, terminal internal carotid, and proximal anterior, middle, posterior, basilar and intracranial vertebral arteries are evaluated. Brightness-(B) mode, intensity/power mode, colour-coded and Doppler mode are used; more recently, motion-(M)mode is also employed. Velocity parameters measured include the Peak Systolic, End-Diastolic and Mean velocities; resistivity parameters include the Gosling's Pulsatility Index and Pourcelot's Resistance Index. For safety, the Thermal Index cannot exceed 6, Mechanical Index 1.9, Spatial Peak Temporal Average Intensity 720 mW/cm². Ultrasound is used to evaluate extracranial and intracranial arterial stenosis, collateral flow as a result of severe stenosis/ occlusion, pulsatile neck masses, subclavian steal, carotid dissection, Takayasu disease, right-to-left shunting, embolisation, intra-operative monitoring during carotid endarterectomy or CABG and post-surgery/stent assessment of stenosis, as well as cerebrovascular reserve to predict stroke risk. It is also used therapeutically to enhance thrombolysis

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(sonothrombolysis). Neurosonology provides an invaluable tool for the clinician in the management and prevention of ischemic stroke.

INTRODUCTION

Stroke is a leading cause of death and disability in many parts of the world. With the increasing numbers and aging of the population, as well as economic transition of less developed to more developed status, the number of people suffering a stroke will likely rise [1]. Approximately 54 – 85% of strokes are ischaemic in nature, with varying mechanisms including large artery atherosclerosis (includes atherothromobosis, artery-to-artery embolism and hypoperfusion), small artery occlusion, cardioembolism, other known causes, or unknown [2]. Large artery disease is largely amenable to detection by ultrasonography.

WHY ULTRASOUND

Ultrasonography is widely used in many fields of Medicine. It is increasingly being used in the diagnosis and management of ischaemic stroke. It is non-invasive, safe, painless, portable, quick, inexpensive, repeatable and reasonably accurate compared to other imaging modalities [3]. Computed tomographic angiography(CTA) and magnetic resonance angiography (MRA) should be considered as complementary rather than competing techniques. CTA requires the availability of a higher-end CT scanner, exposure to radiation and the injection of a contrast medium. MRA requires the availability of an MR scanner, is contraindicated in those with metallic implants or pacemakers, is a challenge in claustrophobic or very obese individuals or those who are haemodynamically unstable or on ventilatory support or restless, and has a long imaging time. The ‘gold standard’ remains catheter angiography with its antecedent risks of radiation, peri-procedural stroke, need for contrast injection, invasiveness and the need for expertise to perform it. None of these techniques is portable; they do not give real-time information; doing frequent, repeated studies and studies at the bedside is thus not possible or feasible. Cost-wise they are far more expensive than ultrasound, in terms of equipment purchase and maintenance and operating costs (Table 1).

Table 1. Comparison of neurovascular imaging methods

Features	Ultrasound	Computed Tomography Angiogram	Magnetic Resonance Angiogram	Catheter Angiography
Accuracy	++	+++	++	Gold standard
Low cost	+++	++	++	+
Speed	++	+++	++	+
Portability	++	0	0	0
Non-invasiveness	+++	+++	+++	0
Lack of radiation	+++	0	+++	0
Lack of contra-indications	+++	++	+	+
Low risk	+++	++	++	+

CLINICAL CEREBROVASCULAR ANATOMY AND PHYSIOLOGY

The brain receives blood supply containing oxygen and the only substrate it can use glucose via the carotid and vertebral arterial systems. The right common carotid artery (CCA) is a terminal branch of the brachiocephalic trunk (BCT, also called the innominate artery) which rises from the aortic arch in the thorax; the other terminal branch is the right subclavian artery (ScA). The left CCA and ScA arise directly and independently from the aortic arch. The diameter of the CCA is approximately 6mm.

The jugular vein on each side often lies superficial to its respective CCA, and can be identified by its easy compressibility, presence of valves, and flow in the opposite direction to the CCA. Each CCA ascends cephalically and bifurcates in the mid-neck usually at the level of the thyroid cartilage into the internal (ICA) and external (ECA) carotid arteries. The ICA then enters the petrous temporal bone.

The ICA can be differentiated from the ECA by a number of features – posterior-lateral location, dilatation at origin, larger diameter, low resistance flow pattern; the lack of branches is the key differentiating feature as is the Doppler waveform response to superficial temporal artery tapping (Table 2).

Table 2. Differentiating internal and external carotid arteries by ultrasonography

Feature	Internal Carotid Artery	External Carotid Artery
Location	Posterolateral	Anteromedial
Dilation at Origin	Yes	No
Diameter	Larger	Smaller
Branches in the neck	No	Yes
Flow pattern	Low resistance	High resistance
Response to superficial temporal artery tapping	No	Yes

The first branch of the ECA is usually the superior thyroid artery. The ECA's branches form many anastomoses with the ICA – superficial temporal artery with the supraorbital artery which is a branch of the ophthalmic artery (OA); facial artery with the dorsal nasal artery which is another branch of the OA.

The vertebral artery (VA) arises from the second part of the ScA and runs medially to enter the transverse process of the sixth vertebra. Each VA then ascends cephalically in the transverse foramen where it is easily and reliably identified, before it exits after traversing the second vertebra, winds round the transverse process of the atlas and enters the foramen magnum. The diameter of each VA is 2-3 mm, and may be dominant in one side, usually the right.

In its course through the petrous temporal bone, the ICA enters the cavernous sinus, runs anteriorly and then loops back, forming the carotid siphon (CS) where the OA is given off – OA flow is towards the eye. The ICA then again ascends cephalically, gives off the posterior communicating artery (PCoMA) which joins the posterior cerebral artery (PCA), and terminates as a bifurcation into the middle cerebral artery (MCA) and anterior cerebral artery (ACA).

The MCA runs laterally (M1 segment) before bifurcating or even trifurcating in the Sylvian fissure (M2 segments). The ACA runs medially (A1) and has a branch to the other ACA called the anterior communicating artery (ACommA), after which it becomes the A2 segment.

The VA in its intracranial portion gives off the posterior inferior cerebellar artery (PICA) and then joins the other VA at the ponto-medullary junction to form the basilar artery (BA). The BA runs cephalically over the pons and terminates at the midbrain-pons junction as the two PCAs. Each PCA runs laterally (P1 segment), is joined by the PCommA after which it becomes the P2 segment and then curves and runs posteriorly.

The Circle of Willis lies at the base of the brain and runs around the pituitary gland. It comprises the distal intracranial ICAs, M1s, A1s, ACommA, PCommAs and P1s, an important intracranial collateral circulation that is complete in only 50% of people.

Approximately 15% of the cardiac output, or about 750 ml/min of blood, is supplied to the adult brain, or 50-55 ml/100g brain/min – it is 75 ml/100g/min in grey matter and 45 ml/100g/min in white matter. Each CCA carries approximately 300-350 ml/min, each VA 50-100 ml/min. Cerebral autoregulation maintains blood flow within a constant range at systemic blood pressures between 50 and 150 mmHg. This is largely achieved by vasodilation and vasoconstriction of cerebral arterioles. The first response to reduced cerebral perfusion is cerebral vasodilation. In severe ischaemia, vasodilation is maximal; the inability for further dilation is referred to as a loss of cerebrovascular reserve, a portend of further ischaemic cell injury and stroke due to hypoperfusion.

IMAGING MODALITIES, INSTRUMENTATION AND INDICES

Extracranial Imaging usually studies the CCAs including the RCCA origin off the BCT, carotid bifurcations, proximal and mid-ICAs and ECAs, and proximal and vertebral portions of the VAs including their origins off the ScAs. The structural integrity and anatomy of the vessels, intimamedial thickness (IMT), plaques, intimal flaps, and luminal thrombi are imaged using B- (= Brightness) mode. Intensity-, also called power-, mode imaging detects the presence of flow. Colour-coded Doppler imaging gives information of the direction of flow and a qualitative estimate of velocities of moving red blood cells. Doppler imaging transforms frequency shifts to velocities; pulse-wave Doppler is preferred over continuous-wave Doppler as specific depths can be targeted and interrogated. The combination of B-mode and Doppler is called Duplex Imaging; the addition of colour-coding makes it Triplex Imaging.

Due to poor resolution, B-mode is used in Transcranial Imaging (TCI) only to locate the midbrain after which Triplex Imaging is performed to locate arteries and determine flow direction and velocities. Intensity-, also called power-, mode imaging is also used. Transcranial Doppler (TCD) is a blind technique using only Doppler to detect arteries and determine flow direction and velocity at various depths. B-mode has been used to detect large intracranial hemorrhages and hydrocephalus, but CT and MRI are far superior techniques. TCD has also been used to detect high intensity transient signal (HITs) presumed to be due to microemboli and to assess intracranial flow changes after manoeuvres. M- (=Motion) mode is a new TCD technique that allows multiple depths to be studied simultaneously. In addition to

detecting areas of stenosis, TCI and TCD also detect the intracranial effects of extracranial disease including collateral flow, and TCD is used to detect embolisation from a more proximal source, and assessment of cerebrovascular reserve. They are also used therapeutically to enhance thrombolysis (sonothrombolysis).

Doppler frequencies of 5 to 10 MHz are used for extracranial imaging as the arteries are superficial; linear array probes are used, with higher frequencies allowing better spatial resolution. Transcranial imaging requires 2 to 2.5 MHz frequencies due to the attenuation by the bones of the skull and deeper location of structures to be imaged; sector or small hand-held probes are used. Duplex and Triplex machines are large and may be portable if wheel-mounted; laptop and even hand-held versions are now available. Pure TCD machines are smaller, and may even be small enough to be carried in a back-pack.

Velocity parameters measured include the peak systolic (PSV), end-diastolic (EDV) and mean (MV) $[= EDV + (PSV-EDV)/3]$ velocities. Resistivity parameters include the Gosling's Pulsatility Index $[= (PSV-EDV)/MV]$ and Pourcelot's Resistance Index (RI) $[= (PSV-EDV)/PSV]$. A number of factors affect velocities, including age, heart rate, blood pressure, haematocrit, fever, hypoglycemia, and hypercarbia.

SAFETY

Ultrasound may potentially harm body tissues due to the high intensity of ultrasound energy used and prolonged duration of insonation, causing thermal (heating) and non-thermal (cavitation, streaming) effects. Heating affects the integrity of tissues and proteins, and the rate and equilibrium of chemical reactions. The Thermal Index (TI) is the regulated matrix; the TI in Soft tissue (TIS) and TI in the Cranium (TIC) are the sub-indices of relevance to neurosonology. Cavitation is due to the expansion and contraction (stable cavitation), and the more dangerous collapse (transient cavitation) of gas bubbles during the oscillatory cycle; pressure, force, torque and streaming are due to the force of pressures. The primary regulated metric is the Mechanical Index (MI). Currently the US Food and Drug Administration stipulates that diagnostic ultrasound scanners cannot exceed a TI of 6 and an MI of 1.9; the Spatial Peak Temporal Average Intensity $I(SPTA)$ cannot exceed 720 mW/cm². For ophthalmic imaging, $I(SPTA)$ cannot exceed 17 mW/cm² while MI should be less than 0.23. At all times, the As Low As Reasonably Achievable (ALARA) principle should be used – the lowest energy intensity and shortest study duration that allows a reasonable study [4].

PERFORMING AN EXTRACRANIAL TRIPLEX EXAMINATION

The patient is placed supine with the head partially laterally rotated away from the side to be examined. The gel-covered probe in B-mode is gently placed transversely in the mid-neck over the sternomastoid muscle and the CCA imaged as a 1cm diameter pulsatile structure with a dark (=anechoic) lumen and bright (=echoic) wall (Figure 1). It is then traced proximally to the base of the neck; on the right side, the BCT is also imaged. The probe is then moved cephalically and the carotid bifurcation identified as two circular pulsatile structures (Figure 2). The ICA and ECA are differentiated and the ICA traced as distally as

possible. The probe is then placed at the bifurcation and rotated so as to image the bifurcation in the longitudinal plane (Figure 3). The distal CCA, proximal ICA and ECA are identified. The vessels are traced proximally and distally. The intimal-media thickness (IMT) is measured, usually in the far wall of the distal CCA (Figure 4). Then the intensity/power mode is turned on to confirm flow in all segments of the arteries (Figure 5). The colour-coded Doppler is then used to confirm flow direction and identify areas of turbulence which may represent sites of stenosis (Figure 6). Finally, in the Triplex mode, the Doppler is used with a narrow sample volume placed in the centre of the artery at specific points as well as areas of turbulence, and an insonation angle steered along the direction of flow, not to be more than 60 degrees (Figure 7). The highest velocities are recorded in the proximal and distal CCA; origin, bulb and as distally in the ICA as possible; proximal ECA.



Figure 1. Right common carotid artery, cross-section, B-mode.

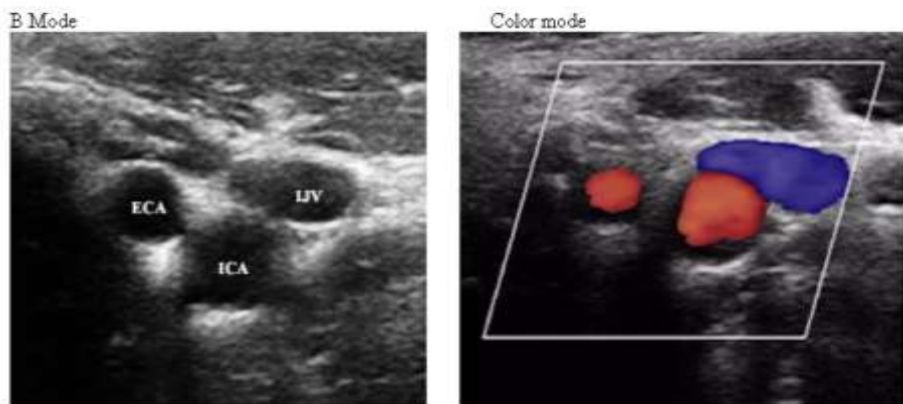


Figure 2. Internal carotid artery, external carotid artery, internal jugular vein, cross-section B-mode (left), colour-coded Doppler mode (right).

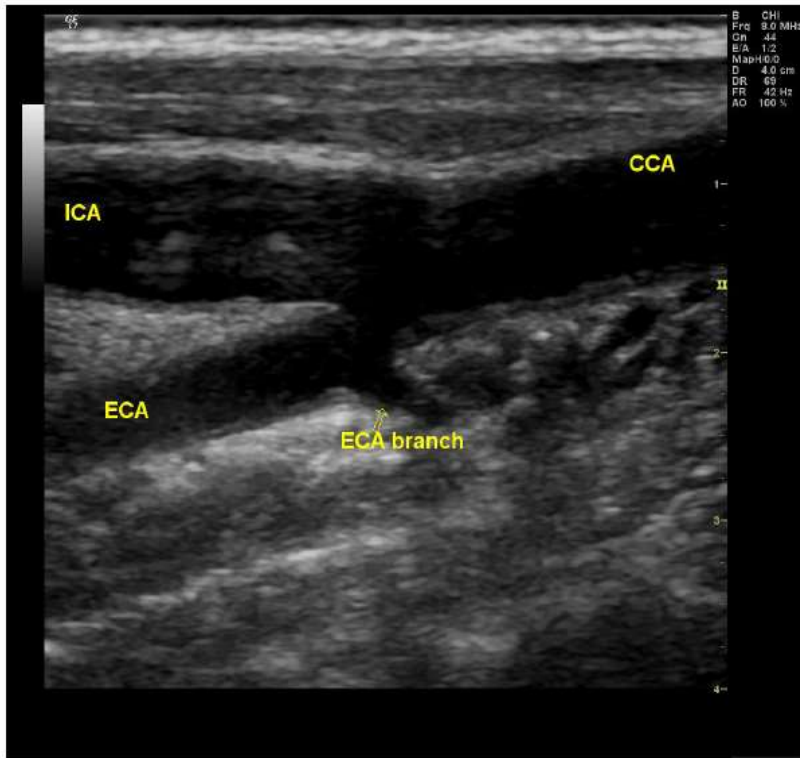


Figure 3. Carotid bifurcation, longitudinal section, B-mode.

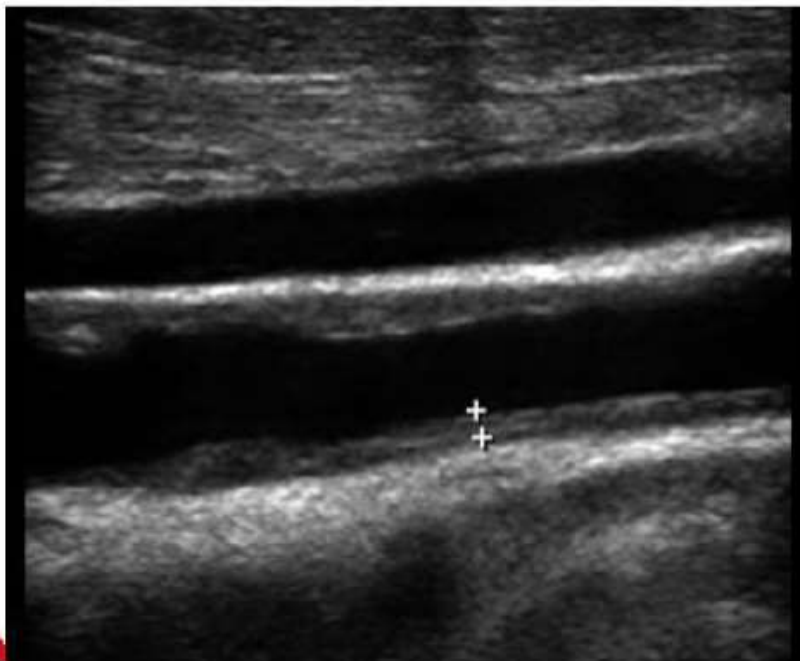


Figure 4. Measurement of intima-media thickness in far wall of mid-common carotid artery.

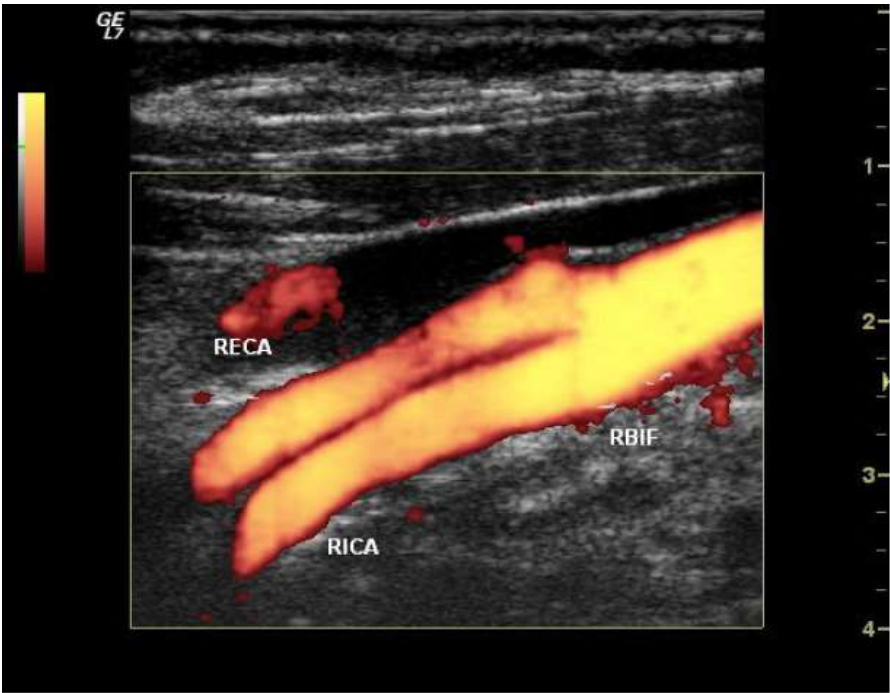


Figure 5. Right carotid bifurcation, longitudinal section, power/intensity mode.

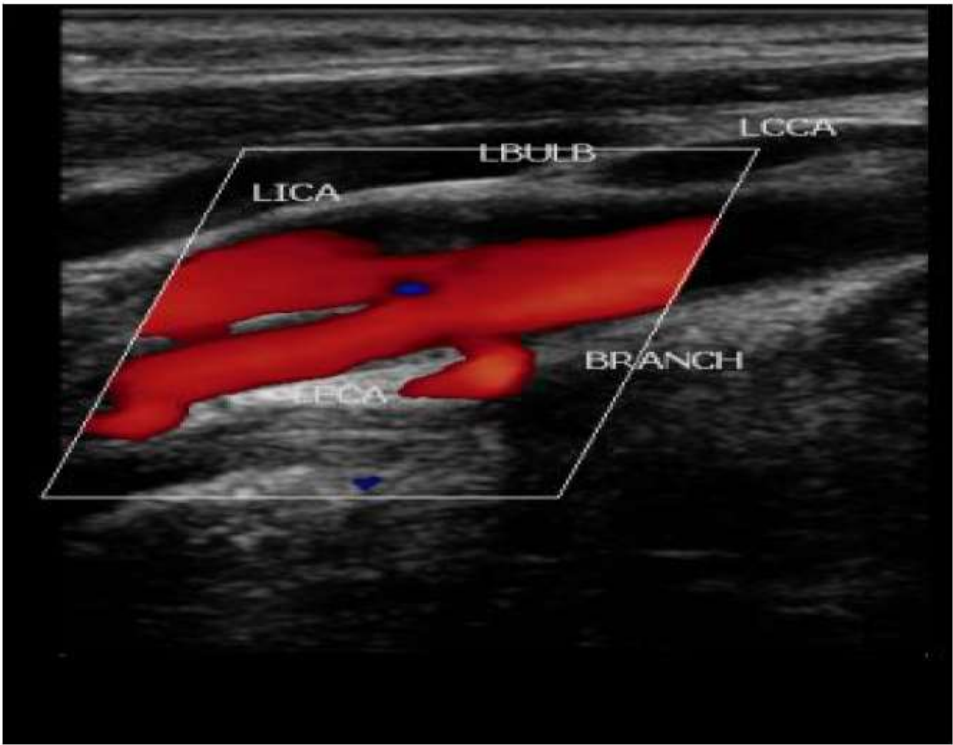


Figure 6. Left carotid bifurcation, longitudinal section, colour-coded Doppler mode.

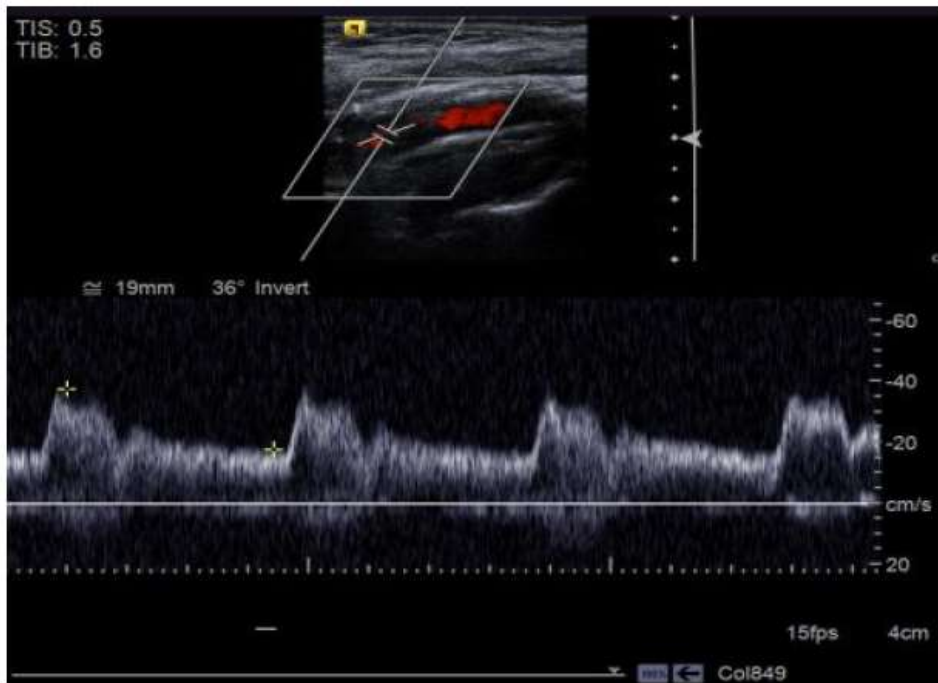


Figure 7. Mid-internal carotid artery, longitudinal section, triplex mode.

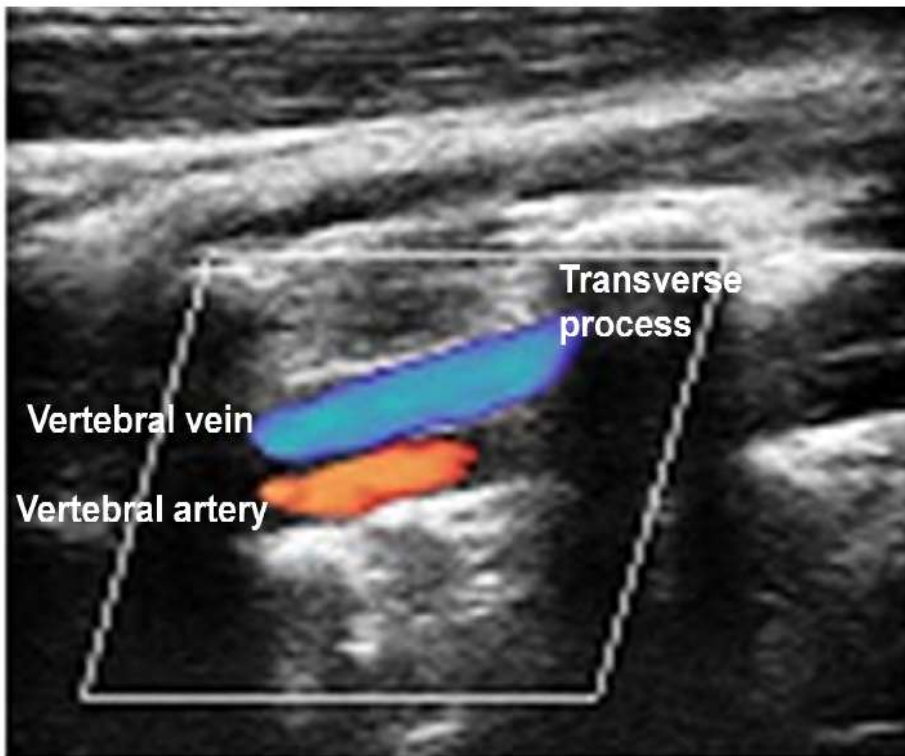


Figure 8. Vertebral artery, longitudinal section, colour-coded Doppler mode.

The B-mode only is then turned on and the probe held vertically over the mid-sternomastoid to identify the VA in its intervertebral segment as a linear structure between the transverse processes of the cervical spines (Figure 8).

The intensity/power, then color-coded Doppler modes are used to study the entire VA including its proximal portions and its origin from the ScA. The Doppler mode is used as for the carotid study, evaluating the mid- and proximal VA and VA origin, as well as areas of turbulence.

The head is then turned the other way and the above processes repeated to study the contralateral carotid and vertebral arterial systems.

Challenges to a successful study include a patient's short and thick neck, high carotid bifurcation, breathlessness, and repeated swallowing.

PERFORMING A TRANSCRANIAL TRIPLEX EXAMINATION

The patient is placed supine, and the gel-covered probe in B-mode is placed gently over the temporal region above the ear in the transverse plane. The probe may be moved back and forth in the temporal window looking for the butterfly-shaped hypoechoic midbrain. Once found, the colour-coded Doppler is turned on and the vessels of the circle of Willis identified (Figure 9). The PCA wraps around the cerebral peduncle. More anteriorly, the MCA travels towards the probe while the ACA travels away.

Often, the contralateral ACA and PCA, and even MCA, may be seen. Areas of turbulence are interrogated by Doppler with the sample volume covering the whole artery to detect areas of significant stenosis, recording maximum velocities in the MCA, ACA and PCA; angle correction is not usually performed. The contralateral side is then examined in a similar manner.

The patient's head is then turned laterally, or the patient is sat up, with the head bent forward. The probe in B-mode in transverse plane is then placed over midline of the nape of the neck posteriorly, just below the occipital nuchae. The foramen magnum is identified as a hypoechoic round structure. The colour-coded Doppler is turned on and the VAs are identified entering the foramen magnum (Figure 10).

At times, the probe has to be placed a little to the side to obtain good images. The VAs are tracked further intracranially where they merge to form the BA which travels further cephalically. The posterior inferior cerebellar artery (PICA) is often identified as a large branch of the intracranial VA. Doppler is used to detect areas of significant stenosis in the VAs and BA.

With the power reduced to 10%, the lightly-gelled probe in transverse plane is gently placed over the eye ball and pointed slightly medially (= orbital window). The colour-coded Doppler is used to detect the OA which flows towards the probe, and Doppler used to determine velocities.

At greater depths, the CS is detected with flow towards then away from the probe. OA and CS velocities are recorded. The contralateral side is then examined in a similar manner.

The challenges include the absence of a temporal window on one or both sides in up to 30% of patients, especially those who are elderly, female or of Asian ethnicity. A restless patient may not allow a detailed examination to be performed.

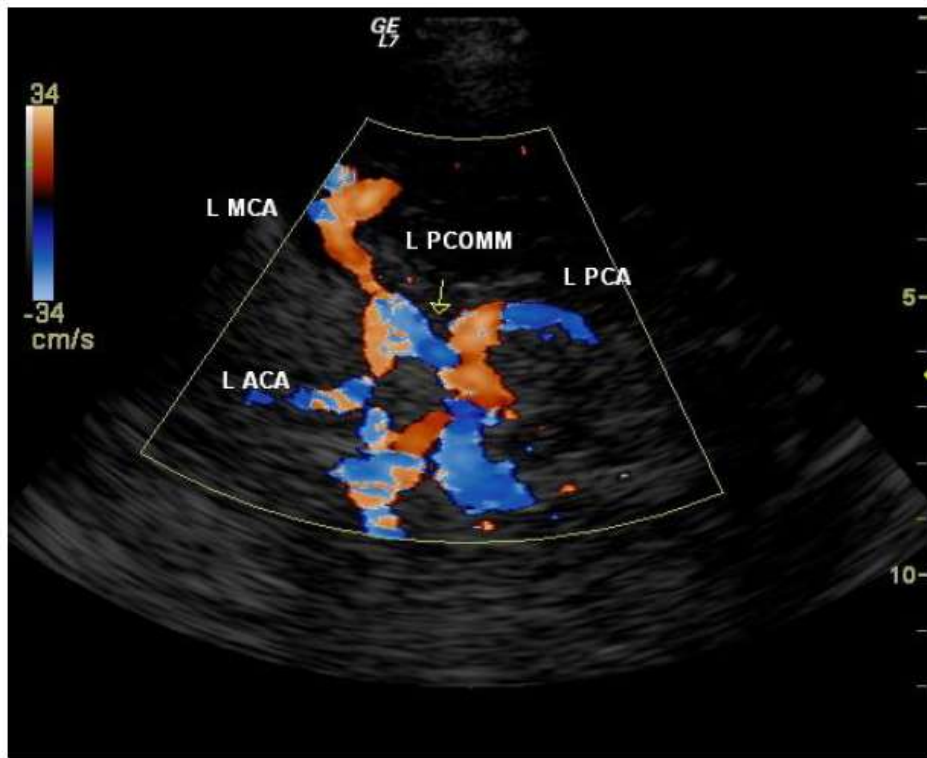


Figure 9. Circle of Willis, left temporal window, colour-coded Doppler mode.

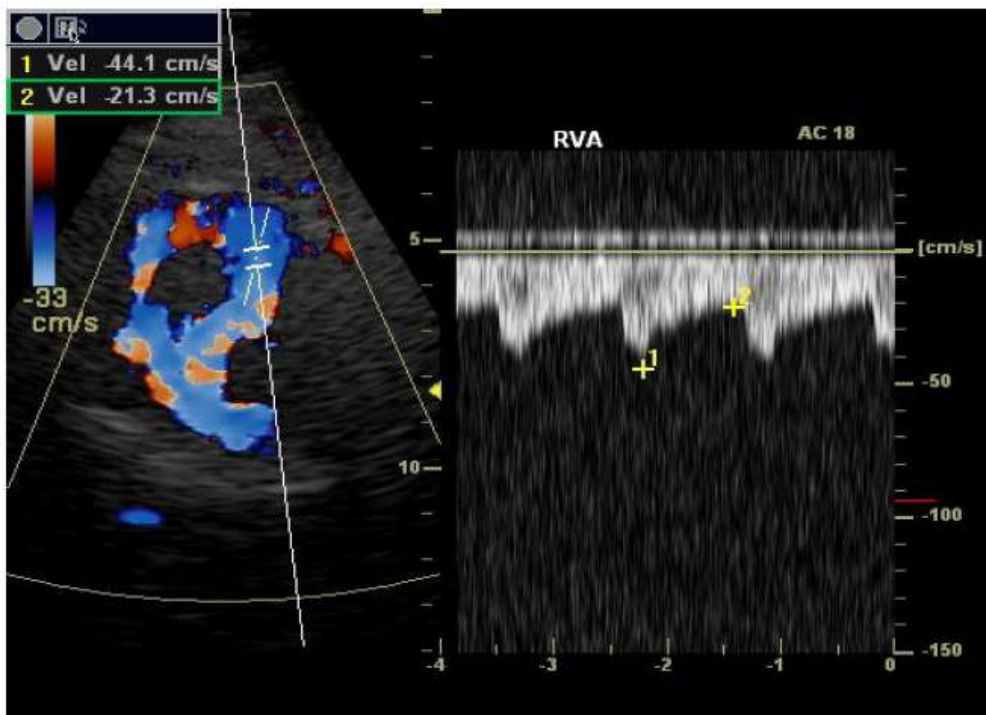


Figure 10. Vertebrobasilar junction, foraminal window, colour-coded Doppler mode.

PERFORMING A TRANSCRANIAL DOPPLER (TCD) EXAMINATION

The patient is positioned as for Transcranial Triplex examination. The gel-tipped small hand-held Doppler probe is placed over the temporal window and pointed inwards and a little anteriorly. The probe is manipulated at various angles to pick up the arterial signal of the MCA. The signal is traced laterally by varying the depth of the sample volume, then again medially till the ICA bifurcation is reached, recognised by MCA flows towards and ACA flows away from the probe. Tilting the probe inferiorly insonates the terminal ICA. The probe is then turned posteriorly and PCA examined. The contralateral arteries are examined in a similar manner.

The probe is then placed over the nape of the neck and the VAs and BA studied by tracking the VA signal through the foraminal window (Figure 11). Finally, with the power reduced to 10%, the probe is placed over the orbital window to investigate OA and CS. Both sides are examined.

Table 3. Identifying intracranial arteries by transcranial Doppler

Window	Artery	Orientation of probe	Depth of insonation (mm)	Direction of flow	Mean velocities (cm/s)
Temporal	MCA	Slightly anteriorly	40 – 65	Towards probe	60
	ACA	Even more anteriorly	65-80	Away	50
	tICA	Inferiorly	60-70	Towards	40
	PCA	Posteriorly	55-80	Towards (P1) Away (P2)	40
Orbital	OA	Slightly medially	40-55	Towards	20 (high resistance)
Window	Artery	Orientation of probe	Depth of insonation (mm)	Direction of flow	Mean velocities (cm/s)
	CS	Slightly medially	60-80	Towards or away	45
Foraminal	VA	If in midline, laterally If lateral, medially	40-90	Away	40
	BA	Midline towards nasion	80-100	Away	45
Submandibular	ICA	Towards ipsilateral eye	40-70	Away	40

The probe may also be placed below the mandible and the distal extracranial ICA insonated on each side in turn.

As TCD is a blind technique, vessels are identified by the window through which they are detected, orientation of probe, depth of insonation, and direction of flow (Table 3). Other challenges are as for transcranial triplex examination, including the absence of a temporal window on one or both sides, and the restless patient.

CLINICAL APPLICATIONS

Ultrasonography has a wide range of clinical applications in the management of ischaemic stroke

Intimamedial Thickness

Increasing intimamedial thickness (IMT) is associated with a moderately increased risk for cerebro- and cardiovascular events [5-10]. Statins statistically reduce the rate of intima-media thickness progression among middle-aged adults with raised LDL-cholesterol and moderate carotid IMT thickening; however, the clinical significance of this reduction is uncertain [11].

In general, IMT exceeding 1mm is considered thickened. IMT is usually measured along the far wall of the distal CCA just before the bifurcation using high-resolution B-mode imaging in the longitudinal section. The first marker is more superficial and is placed at the junction of the (dark) lumen and the (bright) intima. The second marker is placed more deep past the (bright) intima and (dark, presumed) media at the junction of the (dark, presumed) media and (bright) adventitia. Automated systems are available that will provide automatic estimations of IMT.

Extracranial Arterial Stenosis/Occlusion

Extracranial atherosclerosis typically occurs at the carotid bifurcation, possibly due to shear forces due to splitting of blood flows. Internal carotid plaque causes 10-20% of ischaemic strokes [12]. Plaques lead to cerebral ischemia by distal embolisation (of overlying thrombus, platelet clumps, lipid material), or haemodynamic effect of severe stenosis/occlusion leading to distal field ischaemia. Increasing carotid stenosis is associated with increasing risk of ipsilateral stroke and recurrent ichaemia (up to 26% at 2 years), which is reduced by early carotid endarterectomy [13].

Ultrasound is widely used as a screening tool for significant stenosis, and has even been employed as the primary screening tool for clinical trials [14]. Description of the plaque includes mention of the number of plaques, location, size, surface (smooth/ulcerated, the latter carrying higher risk of atheroembolism)), texture (homogenous/heterogenous), echogenicity (hypo-, iso-, hyper-echoic, with respect to the sternomastoid muscle, hypoechogenicity being of higher risk of plaque rupture), calcification (usually casting an acoustic shadow), and intraplaque haemorrhage (seen as a hypoechoic area within a plaque) (Figure 11).

There are a number of criteria that have been used to determine the degree of stenosis [15-17] (Figure 12).

Each neurovascular laboratory is encouraged to develop its own validated criteria for grading stenosis [18].

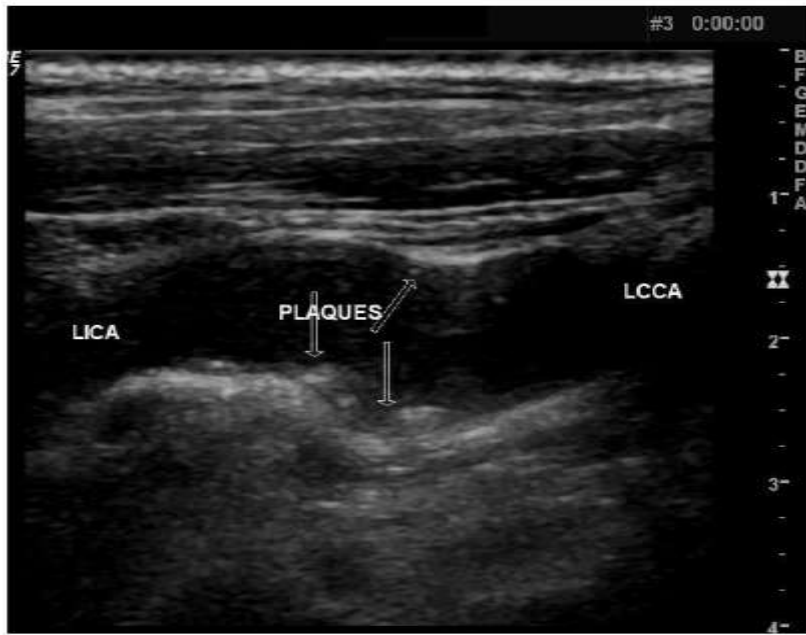


Figure 11. Left carotid bifurcation, longitudinal section, B-mode, showing plaques.



Figure 12. Proximal internal carotid artery, triplex mode, showing severe stenosis.

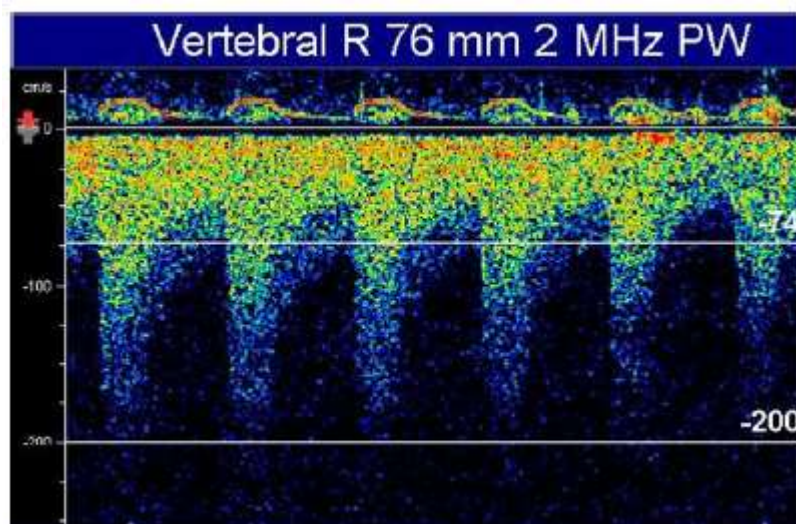


Figure 13. Right vertebral artery, foraminal window, transcranial Doppler, with high velocity showing stenosis.

Atherosclerotic stenosis may also occur at the origin of the VA, and less commonly along the mid-VA (Figure 13). The optimal management of VA stenosis (eg by stenting) is uncertain [19]. Diagnostic criteria for VA stenosis have been proposed [20].

Non-Atherosclerotic Disease - Carotid Dissection, Takayasu Disease, Radiation-Induced Atherosclerosis

Spontaneous carotid dissection causes 2% of all ischemic stroke, 25% among those below 45 years of age. It is due to a tear between the intima and medial layers of the artery, creating a false lumen and intramural thrombosis. Dissection typically starts just beyond the carotid bulb, and may extend intracranially, or at least till the ICA enters the petrous temporal bone [21]. While there is still controversy as to its management by anti-platelets or anticoagulation, making a diagnosis of the cause of a patient's stroke is invaluable; recurrence is uncommon [22]. Ultrasound findings include the detection of an intimal flap (rare), or a double lumen along the ICA beyond the bulb through which blood is able to flow. Most commonly, the ICA is totally occluded just beyond the bulb.

Takayasu arteritis is an inflammatory panarteritis, more frequent in Japan, South Asia, India and Mexico, that causes cerebral ischemia by the haemodynamic effects of progressive stenosis and occlusion of the arteries. The process starts in the aorta and ascends the carotid tree. Treatment includes the use of steroids in the active stage of the disease, and bypass and reimplantation surgery in burnt-out disease. On ultrasound, the inner layers of the arterial walls is diffusely thickened, starting at the proximal CCA, and extending cephalically [23].

The risk of stroke and transient ischaemic attacks is doubled by radiotherapy to the head and neck for malignancies. Radiation-induced atherosclerosis, the thickening of the arterial wall, is probably due to accelerated atherosclerosis due to radiation to the intima-medial layer as well as injury to the vasovasorum of the adventitia [24]. Endarterectomy is difficult to

perform in the scarred tissues; stenting has a high re-stenosis rate [25]. On ultrasound, the atherosclerosis is restricted to the parts of the artery that lay within the field of radiation, with a sharp cut-off in the area of abnormality between the irradiated and non-irradiated parts of the artery.

Subclavian Steal

While usually asymptomatic, the patient may complain of dizziness with excessive use of an upper limb. The pulse and blood pressure may be weaker in that limb. The underlying process is severe stenosis/occlusion of the ScA proximal to the origin of the VA. Severe symptomatic stenosis may be treated by surgery or stenting [26].

On ultrasound, the earliest indication is a transient sharp deceleration of blood flow after the first systolic peak, producing a notch and giving rise to two systolic peaks: the first is sharp, the second is blunt. As the subclavian steal worsens, the systolic notch becomes more obvious and the second systolic peak decreases. The nadir of the notch becomes lower and lower until it reaches and finally crosses the baseline. Flow reversal during systole is initially minimal, but increases until there is complete reversal of flow throughout the cardiac cycle. The steal can be accentuated by the cuff test where a blood pressure cuff is placed over the upper arm, inflated just beyond systolic blood pressure and maintained for a few minutes, and then suddenly completely released (Figure 14).

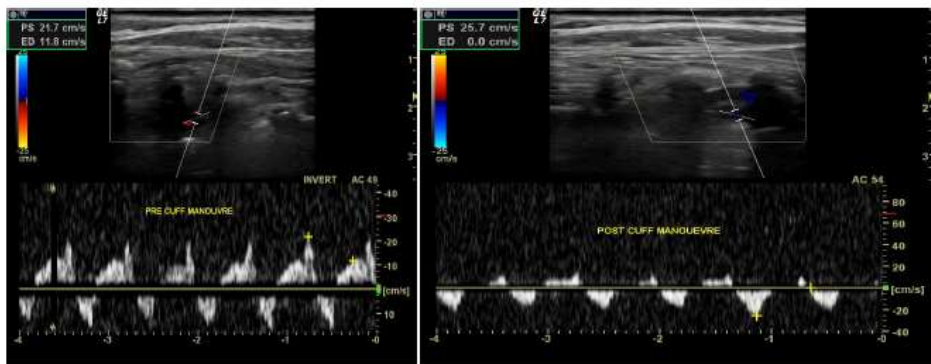


Figure 14. Subclavian steal - alternating flow in vertebral artery accentuated with cuff manoeuvre.

Intracranial Arterial Stenosis/Occlusion

Intracranial atherosclerosis accounts for about 8% of ischaemic stroke in North America and 50% in Asia. The risk of recurrent stroke is 15% per year [27]. Intracranial atherosclerosis typically occurs at sites of arterial bifurcation or merging, or areas of turbulent flow. These sites include the CS, tICA, M1, A1, P1, vertebro-basilar junction, mid-BA, and top of the BA. Optimal management is still uncertain – anti-platelets may be sufficient [28, 29].

Various ultrasound diagnostic criteria have been proposed [30]. Just as for carotid disease, each neurovascular laboratory is encouraged to develop its own validated criteria for grading stenosis.

Collateral Flow As a Result of Severe Stenosis/ Occlusion

Collateral blood flow via extracranial and intracranial anastomoses provides much needed blood supply and reduces the risk of haemodynamic stroke.

In ICA severe stenosis/occlusion between the carotid bifurcation and the origin of OA, extracranial anastomosis between the ECA and ICA via the OA results in increased ipsilateral ECA flow with reversal of the OA flow direction to enter the distal ICA.

Ipsilateral A1 flow is reversed as flow increases in the contralateral A1 and crosses the AComMA to enter the A1; ipsilateral P1 flow may also increase and across the PComMA to enter the distal ICA.

In CCA occlusion, flow may reverse along the ipsilateral ECA to feed the ipsilateral ICA. In VA occlusion, increased flow may occur in the contralateral VA to feed the BA.

Right-To-Left Shunting

Approximately 40% of ischaemic strokes among the young are cryptogenic; more than half have a patent foramen ovale that may provide a conduit for a thrombus from the right-sided circulation to cross into the left-sided circulation and enter the cerebral circulation [31]. Optimal therapy is controversial – closure, anti-coagulants, anti-platelets are used [32].

While the ‘gold standard’ for the diagnosis of PFO is transoesophageal echocardiography (TEE), TCD of the MCA has been shown to have a very high sensitivity and specificity [33]. For those patients with poor temporal windows, the foraminal window with the insonation of the BA can be used [34].

There is a consensus recommendation for the performance of a ‘bubble-TCD’ for the detection of a right-to-left shunt (RLS). This involves the rapid injection via a large bore needle placed in the antecubital region of a well-agitated mixture of 9 ml of saline and 1 ml of air, while insonating the MCA.

The presence of an RLS is indicated by the swift occurrence of high intensity transient signals (HITS) presumed to be microbubbles (MB) - 0 MB (negative result); 1-10 MB; >10 MB with no curtain, and curtain [35]. The optimal position may be with the patient sitting and test performed with the Valsalva manoeuvre [36]. Approximately 10% of RLS are extra-cardiac eg pulmonary vascular malformations.

Embolisation

The presence of HITS gives a 7 to 13-fold increase in the risk of recurrent stroke [37, 38]. HITS are attributed to microemboli from a more proximal source – thrombi (eg from atrial fibrillation, prosthetic cardiac valves, atherosclerotic plaques extra or intracranially), gas

bubbles (eg from prosthetic cardiac valves). They are reduced by double-antiplatelet therapy, but reduction in clinical events has not been shown [39, 40].

The TCD probe is fixed on a head frame and the artery closely monitored, preferably for at least 30 mins, on at least 3 occasions. There are consensus criteria for Doppler diagnosis – transient signal usually lasting less than 300 milliseconds, amplitude usually at least 3 dB higher than that of the background flow signal, signal is unidirectional within the Doppler spectrum, and signal is accompanied by an audible “snap,” “chirp,” or “moan” [41].

Intra-Operative Monitoring during Carotid Endarterectomy (CEA), Coronary Artery Bypass Grafting (CABG)

Intracranial HITS are frequently seen during CEA, especially during the mobilization of the carotid artery and during cross-clamping, and even more-so after release of the cross clamps after arterial wall closure. These are presumed to be due to emboli from the atherosclerotic plaques, or even air that entered the surgical site during surgery.

Similarly, HITS are seen during CABG. These may be due to gaseous bubbles from the heart-lung machine or atherosclerotic material during reversed flow along the aorta.

During CEA, due insufficient intracranial collaterals, MCA flow may fall precipitously during ipsilateral cross-clamping – some surgeons would insert a shunt across the surgical incision site in this setting. Monitoring is achieved by using a head frame holding the monitoring probes.

Post-Surgery/Stent Assessment

Patency of the carotid arteries immediately after CEA or stenting, intra/post-operative carotid dissection, in-situ thrombosis, are detected by ultrasound, which may require re-surgery/stent. The occurrence of subsequent re-stenosis some time after surgery/stenting is also easily assessed. If a patient develops post-procedure ipsilateral cortical signs, the finding of extremely high ipsilateral intracranial velocities may support the diagnosis of hyper-perfusion syndrome, necessitating blood pressure reduction.

Cerebrovascular Reserve to Predict Stroke Risk

Vasodilation is one of the cerebrovascular responses to cerebral ischaemia so as to maintain blood flow when there is blood flow reduction. Carbon dioxide (CO₂) is a potent cerebral vasodilator. A reduced or failed vasodilatory response to CO₂ would indicate that vasodilation is maximal, no more vasodilation is possible ie there is absent/reduced cerebrovascular reserve (CVR). Reduced CVR is a marker for recurrent cerebral ischaemic events, and may be used to select patients for superficial temporal artery-middle cerebral artery bypass and carotid surgery [42].

The breath holding index (BHI) is one method of assessing CVR. It is determined by measuring the mean velocity before and after breath-holding: $BHI = (\text{velocity at end of breath-holding} - \text{velocity at start of breath-holding}) \times 100 / (\text{velocity at start of breath-holding})$

x number of seconds of breath-holding). A BHI < 0.69 indicates an increased risk of ipsilateral ischaemic cerebrovascular events [43].

Sonothrombolysis

Ultrasound is able to assist in the lysis of blood clots by a number of mechanisms, including clot agitation to break up clots and thus increase the surface area for natural or administered thrombolytic molecules or agents to act. The administration of ultrasound during the administration of systemic thrombolytic agents has been shown to improve recanalisation, without harm or benefit on death or dependency at 3 months [44].

The ultrasound probe is fixed to the head by a frame with the ultrasound beam focused at the site of occlusion for an hour. Recanalisation is heralded by the return of flow velocities to a more normal level at which time the insonation can be terminated.

PHYSICIAN ACCREDITATION

The performance of a competent neurosonological examination will greatly assist in patient care. There are a number of international accreditation examinations available for physicians held a number of times in the year in various cities. They are offered by the World Federation of Neurology Neurosonology Research Group as well as the American Society of Neuroimaging [45, 46].

CONCLUSIONS

Ultrasound is a safe, useful and versatile technique in the management of patients with cerebrovascular disease, not just for diagnosis but potentially also for treatment (sonothrombolysis).

Further research and clinical trials will provide more information as to the role of neurosonology in the management of ischaemic stroke.

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Chapter 54

MODELING AND OPTIMAL DESIGN OF POWER HIGH STROKE PIEZOELECTRIC ACTUATORS FOR ROTORCRAFT APPLICATIONS

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ABSTRACT

Because of helicopter main rotor is a source of external noise, cabin noise and intensive vibrations, the problem of active vibration preventing and suppression attracts attention of many engineers and scientific investigators last three decades. The mentioned vibrations are caused by the non-symmetrical and unsteady rotor aerodynamics. Last years to realize the advanced helicopter rotor control the Active Trailing Edge (ATE) or Active Flap (AFC) concepts have been proposed. According to AFC concept the small flap which is placed on the distance close to 0.7 of the blade radius, can deflect upwards or downwards, and due to this the blade changes the aerodynamic properties, and vibration level can be decreased.

Very effective and structurally simple solution for driving whole blade or active flap, is the use of power piezoelectric devices. Such piezoelectric actuator drives an active flap hosted on the helicopter rotor blade to change its aerodynamic properties at rotor rotation. As the flap deflection properly synchronized with the azimuthal angle of rotor blade, such active flap control devices allow us to significantly reduce the noise and vibration level of helicopter.

Despite the inherent ability to create of very high forces, all piezoelectric actuators have some drawbacks, among which an incapability to produce enough large displacement, and bad withstanding an external tensile stress. These limitation is forced to develop the different structures for the stroke amplification. This article concern a structural optimization problem for a flexensional piezoelectric actuator which consists

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of the high power piezoelectric stack and polymeric composite shell intended for amplification of the stroke. To formulate an optimization problem we parametrize the shape of the amplifier's shell by the rational Bezier curves, which parameters (coordinates and weights of the control points) are changed iteratively by genetic algorithm according to the fitness function value calculated by the finite element model of the transducer with varied geometry. We used the fitness function as the shell's deflection under action of external force. Finally, we demonstrate some optimization results, which confirmed the proposed approach.

1. INTRODUCTION

Reducing high frequency dynamic structural loads that increase the wear of components by minimizing the corresponding disturbances, is an important aim for aerospace designers [1-3]. Besides, the modern aircraft must meet the strong requirements to limits for emitted noise. The helicopter vibrations are mainly excited by periodic forces generated by the main rotor and transferred through the rotor hub and the gearbox into the fuselage. Most intensive vibrations are generated at forward flight and because of the blade-vortex interaction (BVI). At the forward flight with the airspeed V the chordwise velocity U of a blade element is given by

$$U = \Omega r + V \sin \psi, \quad (1)$$

where ψ is the blade azimuthal position, Ω is the rotor angular velocity, and r is the blade element distance from the rotor axis. On the retreating side the component of rotational velocity and horizontal velocity are subtractive and a region of reversed flow will exist. Since the flow in the reversed flow region is from trailing edge to leading edge, it has the effect of pushing the rotor around the mast thereby extracting power from the airflow. BVI is an example of an intense vertical velocity field with high velocity gradients. While passing an airfoil at a predetermined distance, a convecting vortex of positive circulation produces a downwash velocity while upstream of the blade (airfoil), and this changes to an upwash as it moves downstream. This situation leads to a rapidly and continuously changing angle of attack, resulting in highly unsteady aerodynamic loads [4].

Different approaches have been explored to achieve a reduction of the helicopter vibration. Passive means, either located in the rotating or fixed system, aim at exclude the transfer of the exciting forces into the fuselage by especially tuned absorbers. Research activities of last 40 years showed the high potential of active rotor control with a higher harmonic blade pitch variation for vibration reduction [4]. In this case a blade pitch oscillation with harmonics of the rotor rotational frequency is superimposed to the collective and cyclic blade pitch (see Figure 1, a). The Higher Harmonics Control (HHC) provides that the control signal was fed into actuators located beneath the swashplate. The promising theoretical and experimental results led to the development of the Individual Blade Control (IBC) system (see Figure 1, b), where the original pitch links located above the swashplate are replaced by power actuators which change the incidence (or pitch) angle of the blades. Both technologies work with a higher harmonic excitation of the blade pitch at the blade root, which is a very energy-consuming, and also can lead to the blades excitation on first twisting

vibration mode [3]. With an active trailing edge (ATE) flap the excitation is not introduced at the blade root, but localized at a distance 75 – 90% of the blade radius (see Figure 2). ATE concept can be implemented in the form of flexible or turned trailing edge flap, and as the locally morphing airfoil [8].

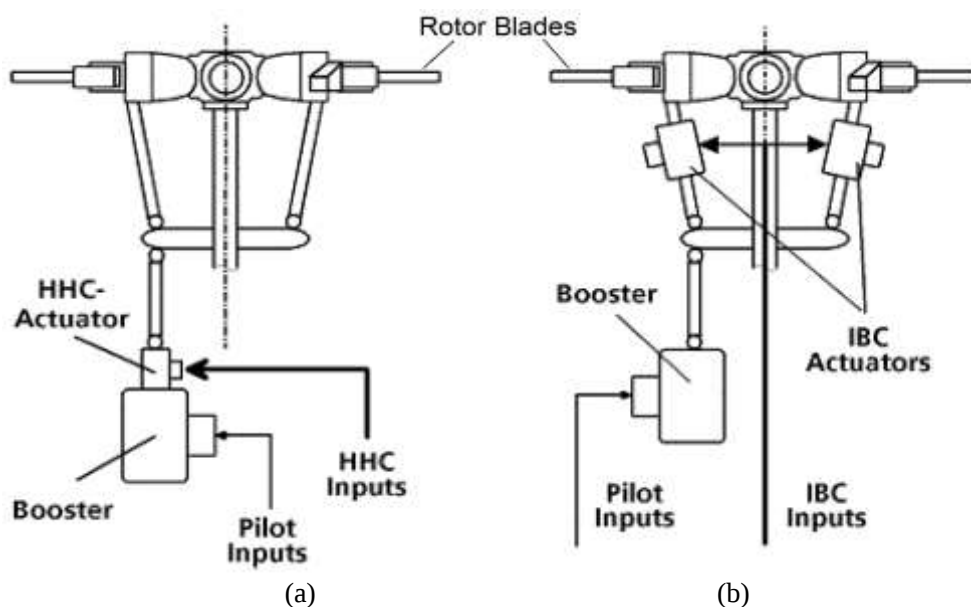


Figure 1. HHC vs IBC concepts [3].

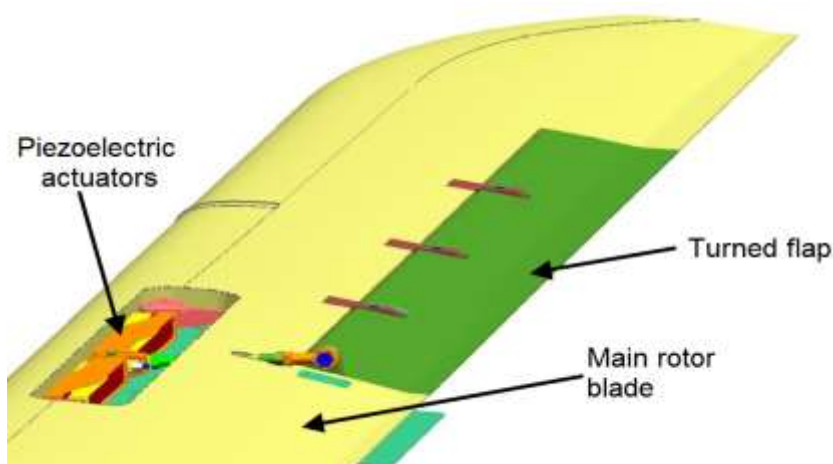


Figure 2. SMART rotor blade with piezoelectric actuator driving the trailing edge flap [6].

The Actively Controlled Flaps are generally 15% of a chord length and most known structures are driven by the power piezoelectric transducers. Due to the high operating forces and frequency, smart materials such as piezoelectric actuators are well adapted for this task, but relatively large displacements require some sort of mechanical amplification of these devices movement. Because of the very small displacements created by the piezoelectric devices some design solutions to amplify the stroke of PZT actuators are proposed [7, 9].

The most important requirements to the piezoelectric actuator for the active flap design are the following [2]:

- i. big force and large displacement in compact sizes,
- ii. high resolution within the micrometer range,
- iii. very short response time less than 1 ms,
- iv. life time greater than 10¹⁰ cycles,
- v. low voltage supply less than 150V DC, no backlash and no play,
- vi. low power consumption when statics,
- vii. severe environment compatibility (broad temperature and moisture ranges).

Among the known structural concepts of actuators most adoptable properties had the flextensional actuator which consist of the high power piezoelectric stack and shell which supply the stroke amplification (see Figure 3).

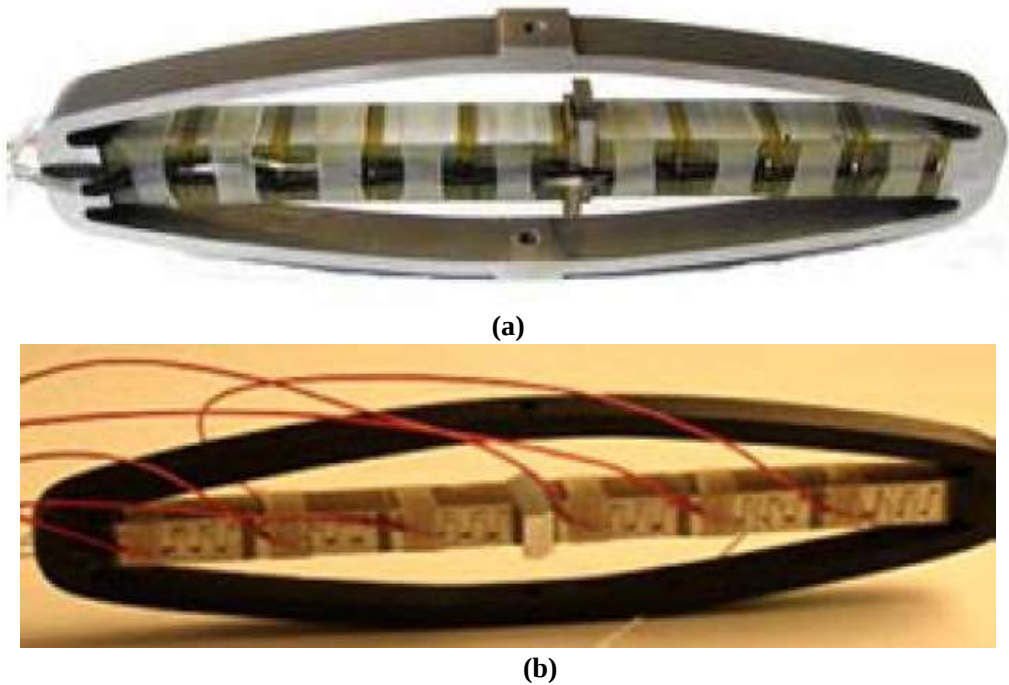


Figure 3. Piezoelectric amplified stack with steel (a) and polymeric composite (b) shell [2].

These actuators provide a high stiffness and relatively large displacements. On the other hand, their mass is not optimized and the metallic shell implies a large penalty on the mass. In order to improve the mass of these flextensional actuators a carbon composite is used instead of metal for the frame. Figure 4 demonstrates the mounting of actuators on the rotor blade and shows the transmission of aerodynamic forces to the actuator through the levers. These forces deflect the flap in opposite direction to its active deflection. Because of acting aerodynamic forces a higher flap deflections require larger and therefore heavier actuators, but additional mass is needed to equilibrate the center of mass of the blade close to active flap mounting.

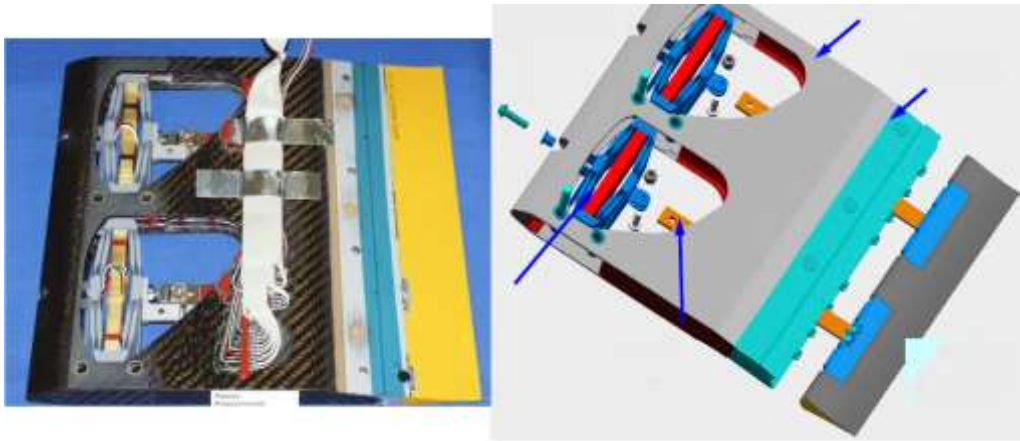


Figure 4. Mounting of active flap and driving piezoelectric actuators on the rotor blade [6].

In order to realize the active flap on the real-world five-bladed helicopter we need to optimize the mechanical properties of the considered flextensional actuator. Primarily we investigate by the finite element method (FEM) the properties of actuator with most widely used elliptic composite shell. Next we describe the shape of the shell by the rational Bezier curves, which allow the good flexibility and to simultaneously optimize the stiffness and operating stroke. An optimization problem will be stated with the given performance of piezoelectric material and the constraints will be imposed on the acting external force as well as the coordinates of the points controlling the Bezier curve behavior. As the fitness function we accept the actuator's stroke at permissible operating voltage on the PZT stack and given external force. Because of total number of degrees of freedom for this problem is very big, we use genetic algorithm implemented in the soft package MATLAB (Genetic Algorithm Toolbox) and simplified one-quarter 2D FEM model of actuator worked in Comsol Multiphysics. Finally we demonstrate the effectiveness of the developed optimization method and significantly improved properties of the optimized actuator.

2. FINITE ELEMENT MODELING OF FLEXTENSIONAL ACTUATOR WITH ELLIPTIC SHELL

We have investigated only actuators with shell made of glass fiber epoxy polymeric composite which had the longitudinal Young modulus $3 \cdot 10^{10}$ Pa and density 1850 kg/m^3 . Technology of the shell manufacturing includes the winding of unidirectional glass-fiber tape on a mandrel with appropriate shape, then curing in autoclave until the complete solidification. The size of the finished shell should provide a prestress for the assembled piezoelectric stack for a double-side acting actuator. Stack was constructed of a multilayer piezoelectric ceramic PZT-5H, the thickness of each layer, polarized in thickness, was 0.5 mm. Driving electric potential was taken as 400 V. Geometry of a typical FEM model is presented in Figure 5.

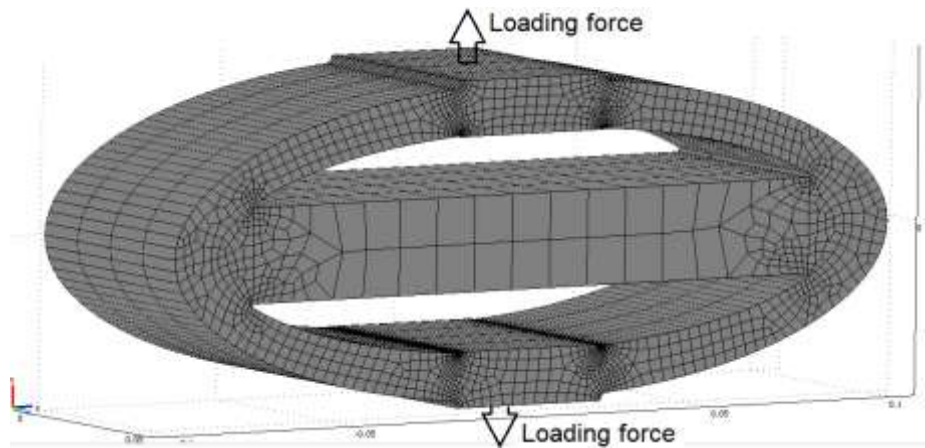
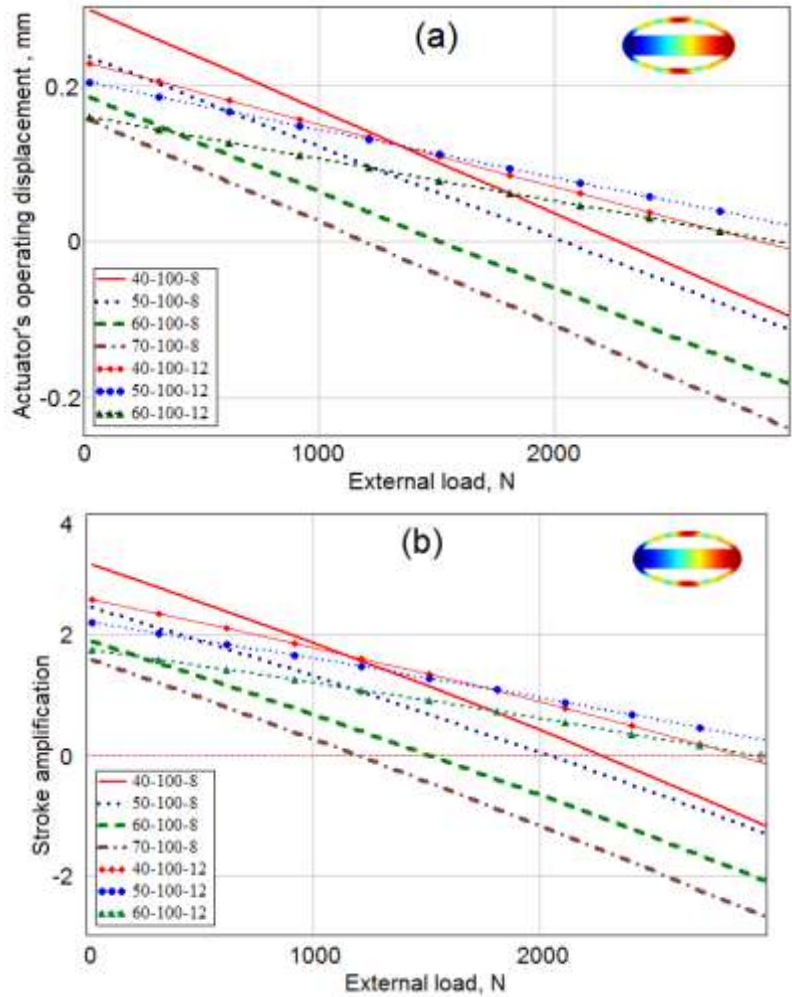


Figure 5. Geometry of actuator’s FEM model with elliptic shell and varying ratio of the ellipse semiaxes.



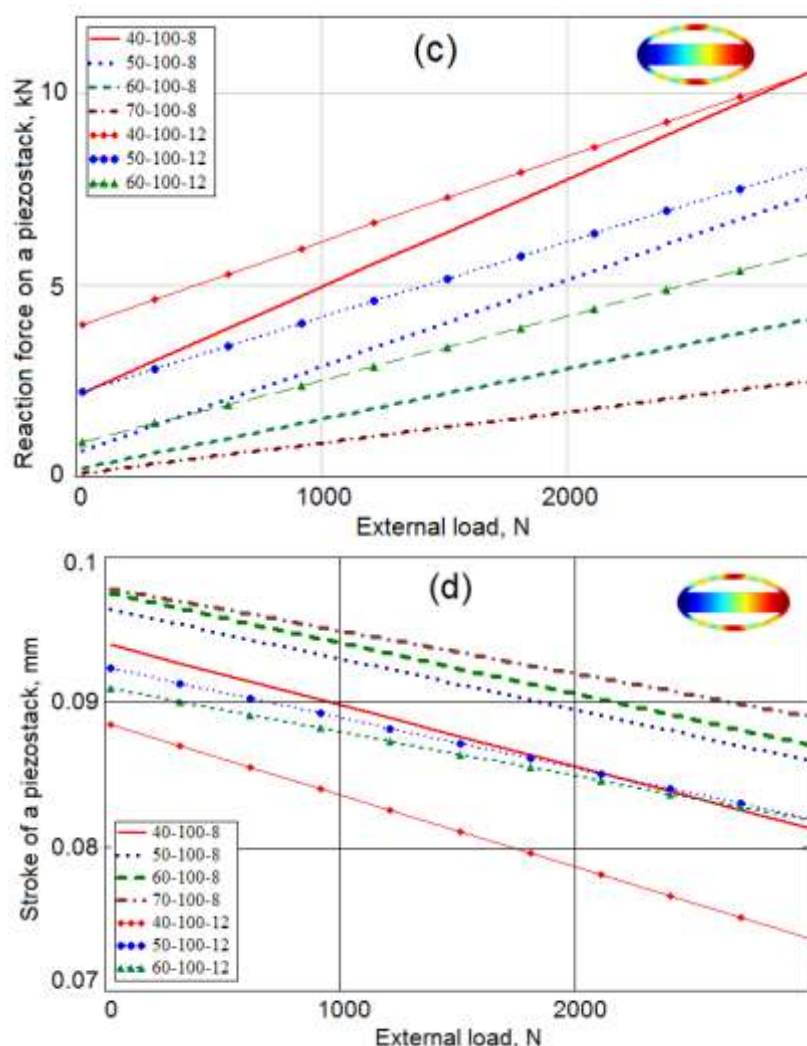


Figure 6. Dependencies of the actuator's stroke (a), amplification ratio (b), and reaction force applied to the PZT stack (c), and compressive deformation of the stack under this reaction force (d) on the external force for the different dimensional parameters of composite shell.

In our numerical experiments we used shell geometry with the thickness 8 mm and 12 mm; major semi-axis was always 100 mm, whereas the small semi-axis took values 40, 50, 60, 70 mm.

The FEM static analysis was performed as follows. After applying of the driving potential the stack lengthened, deforming the shell and causing it to contraction in the vertical direction. Then to the executive surfaces of the shell (small quadrilateral planes) the gradually increasing tensile force was applied. When the structure was loading the values of operating stroke, applied reaction force and deformation of piezoelectric stack were monitored. As soon as the executive surfaces displacement returns to zero, the blocking force is recorded.

Then to the executive surfaces of the shell (small quadrilateral planes) the gradually increasing tensile force was applied. When loading, the values of operating stroke, applied reaction force and deformation of piezoelectric stack were measured. Some simulation results

are presented in Figure 6, where, for example, the acronym 40-100-8 means as follows: semi-major axis of 100 mm, small axis of 40 mm, thickness 8 mm.

One can see from the plots (Figure 6, a, d) that the total compliance of actuator is determined by the shell, not PZT stack that has a greater stiffness. Indeed, total displacement of actuator (40-100-8) at the loading force 2 kN consists of 0.23 mm, whereas deformation of PZT stack is equal to 0.01 mm, only. This can be explained by the results of FEM modeling. Figure 7 shows von Mises stress distribution in shell before and after applying the external tensile force. As one can see the stress-strain state along the shell is non-homogeneous, thus we can enhance the actuator performance by varying the shell thickness distribution.

As might be expected, thicker shell has more stiffness, but lesser free stroke. Dependence of the actuator's parameters on the ratio of the elliptic shell axes is also essential. The more shallow shells provide greater amplification, but lesser ability to counteract external loads. This fact is illustrated in Figure 8, where dependencies of the amplification ratio and blocking force for the different shell dimensions are presented.

These preliminary results justify the need and opportunity to optimize the shell's geometry. This optimization can be performed by varying the shape of the generatrix and thickness distribution of the shell.

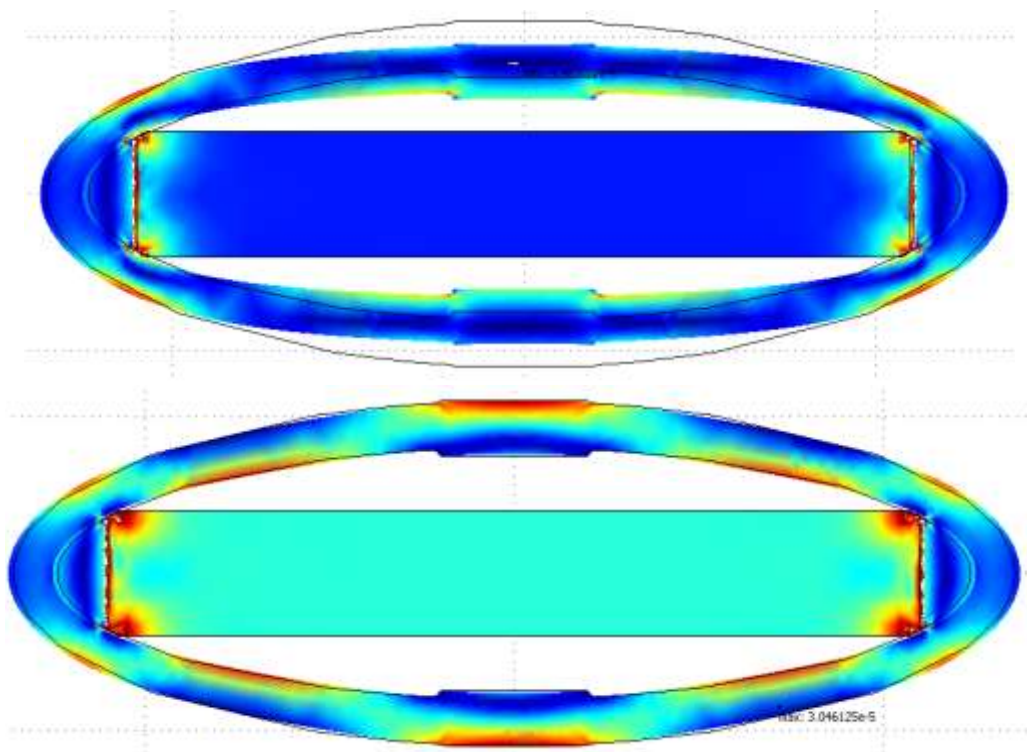


Figure 7. Deformed shape and von Mises stress distribution in polymeric composite shell before (above) and after (below) applying the tensile force to the upper and lower plate of shell (Results of static FEM simulation. Von Mises stress is illustrated by the colour: most intensive red colour corresponds to the value 35 MPa. All deflections are increased in 50 time).

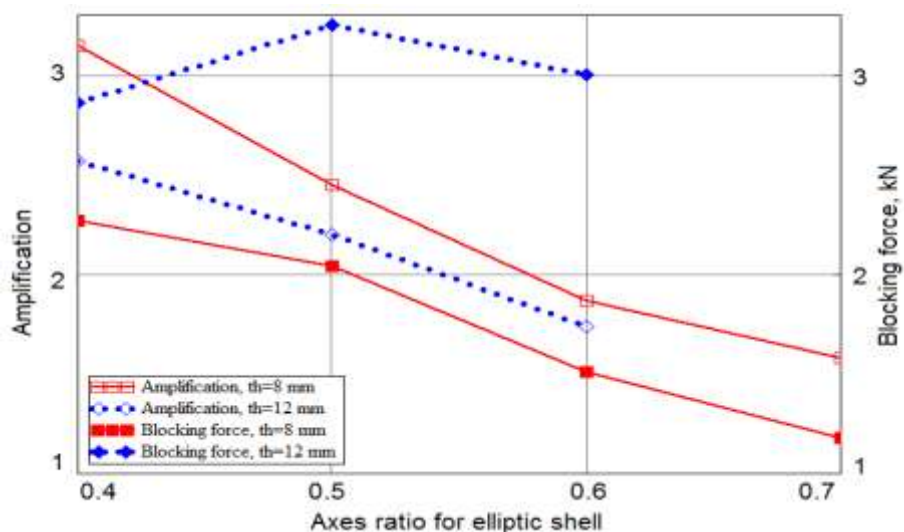


Figure 8. Amplification ratio and blocking force of the flextensional actuator as the functions of the different axes ratio for the elliptic composite shell with thickness 8 mm (solid lines) and 12 mm (dotted lines).

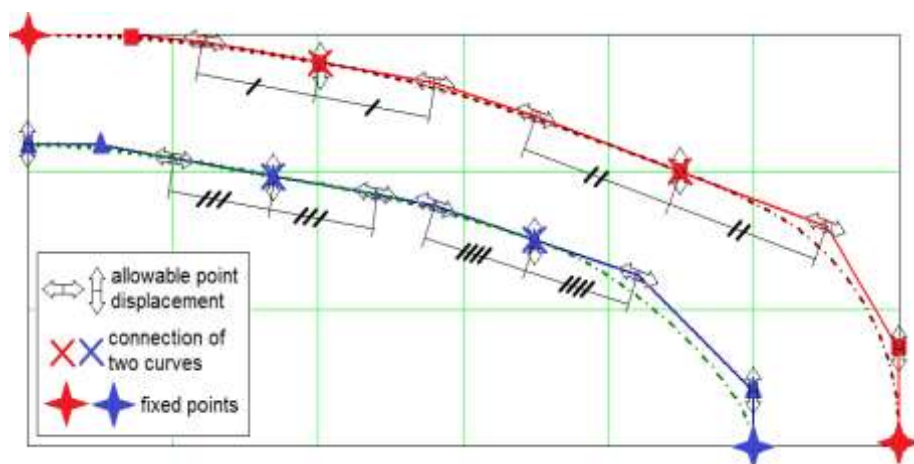


Figure 9. Representation of the shell's profile by the 3rd order rational Bezier curves.

3. GA BASED OPTIMIZATION OF THE SHELL GEOMETRY

As the objective function is advisable to choose the operating stroke h_{op} at the given external load F_{act} (in our case this value is 600 N). To describe the shape of the generatrix with the necessary flexibility we use the rational Bezier curves because of these lines are inherent for the used FEM software Comsol Multiphysics. According to the adopted assumptions the one-quarter of the symmetrical shell can be presented as two branches, each of which consists of three 3rd order Bezier curves (see Figure 9). The rational Bezier curve of n -th order, defined by the $(n+1)$ control points P^i , is described by the equation:

$$R(u) = \sum_{i=0}^n w_i B_n^i(u) P^i \Big/ \sum_{i=0}^n w_i B_n^i(u), \quad (2)$$

where $B_n^i(u)$ are the Bernstein polynomials, defined as

$$B_n^i(u) = \frac{n!}{i!(n-i)!} u^i (1-u)^{n-i}, \quad (3)$$

w_i , $i = 0, 1, \dots, n$ are the weights of the control points, and u is the parameter runs through the values from 0 to 1.

At optimization process, external dimensions of the shell remain to be unchanged, so, only shape of the generatrices is optimized. In order to supply C^1 continuity at the connection points (denoted by “×”) it is necessary to impose additional constraints on the location of the control points, which are adjacent to the point connection of two curves. If P^c is the vector coordinates of connection point, P^{lc} is the vector coordinates of a point adjacent to the left-to-point connection, then vector coordinates of a point adjacent to the right-to-point connection is

$$P^{cr} = 2P^c - P^{lc} \quad (4)$$

Figure 9 shows the equality of the corresponding edges of the control polygon. The placement on the vertical (horizontal) lines of points adjacent to the terminal points (denoted as “+”) of the generatrices allows us to automatically satisfy condition of C^1 continuity due to symmetry of the shell (see Figure 9). So, we have 21 degrees of freedom for the points coordinates, and 18 degrees of freedom for the weights, at last, total number of DoFs is 39.

All control points positions are constrained by the system of inequalities:

$$\begin{cases} X_i > X_{(i-1)}, & i \in [1; (n-1)]; \\ Y_i < Y_{(i-1)}, & i \in [2; n], \end{cases} \quad (5)$$

by the equalities for connection points from the relationship (4)

$$\begin{cases} X_0 = 0; & Y_0 = b^{in,out}; \\ Y_1 = b^{in,out}; \\ X_n = a^{in,out}; & Y_0 = 0; \\ X_{n-1} = b^{in,out}, \end{cases} \quad (6)$$

where the dimensions of the external generatrix a^{out}, b^{out} are fixed, but the dimensions of the internal generatrix are satisfied by the relationships:

$$\begin{cases} a^{out} > a^{in} = \text{fixed} \\ b^{out} > b^{in} = \text{varied} \end{cases} \quad (7)$$

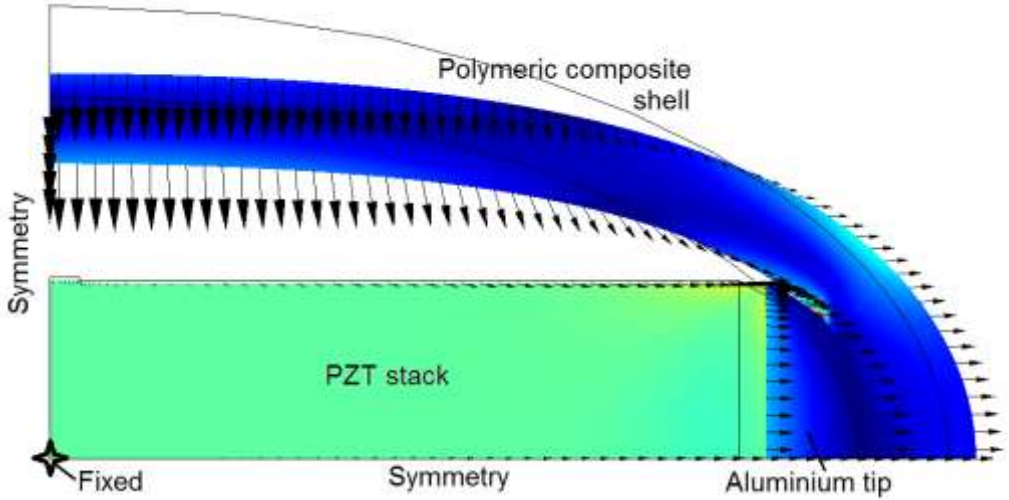


Figure 10. Geometry and displacements field for optimized FEM model of the flextensional actuator.

All weights also are constrained by the system of inequalities. Each of the three Bezier curves, which form the generatrix, is described by its own equation (2). But to ensure the smoothness of generatrix, end points of one curve and the initial point of the connecting curve have the same weight. So, we have the following system of constraints for weights:

$$\begin{cases} w_0 = 1; \\ 0 < w_i < 5, \quad i \in [1; n]. \end{cases} \quad (8)$$

After a number of numerical simulations some additional restrictions are introduced to narrow the range of the coordinates of control points

$$\sum_{k=1}^i \xi(k, a^{in,out}) - 0.25 \xi(i, a^{in,out}) < X_i < \sum_{k=1}^i \xi(k, a^{in,out}) + 0.25 \xi(i, a^{in,out}), \quad (9)$$

where $\xi(i, a^{in,out}) = \frac{a}{372} [64 - (i-1)^2]$ $i = 1, \dots, n-1$.

These constraints allow us to agree the density of the control points with the curvature of the generatrix, and thereby significantly reduce the search area. This is very important for a problem with large number of DoFs. Similar optimization problems can be successfully solved only by the genetic algorithm. Therefore we have used the Genetic Algorithm Toolbox MATLAB which has the advanced optimization means and direct access to the FEM computation.

To reduce the computational cost, our FEM model was very simplified. Due to symmetry of the shell we consider one-quarter of actuator's structure (see Figure 10). All material

properties and displacements are considered as linear. Quadrilateral FEM mesh consists of near 500 elements. FEM model works in the Structural Mechanics – Piezoelectric mode. At each iteration step, GA Toolbox rebuilds the shell geometry, remeshes it, and performs the parametric static analysis. The calculated value of the actuator stroke is passed to the GA Toolbox, which changes the value of the design variables according to the constraints. Whole computation flowchart is controlled by MATLAB which in turn refers to modules that perform the parametric finite element analysis. These modules are implemented as standard MATLAB's m-files. Most important GA settings are: population size is 50, elite count is 5, crossover is configured as scattered, mutation – as adaptive feasible, used hybrid function is “fmincon”.

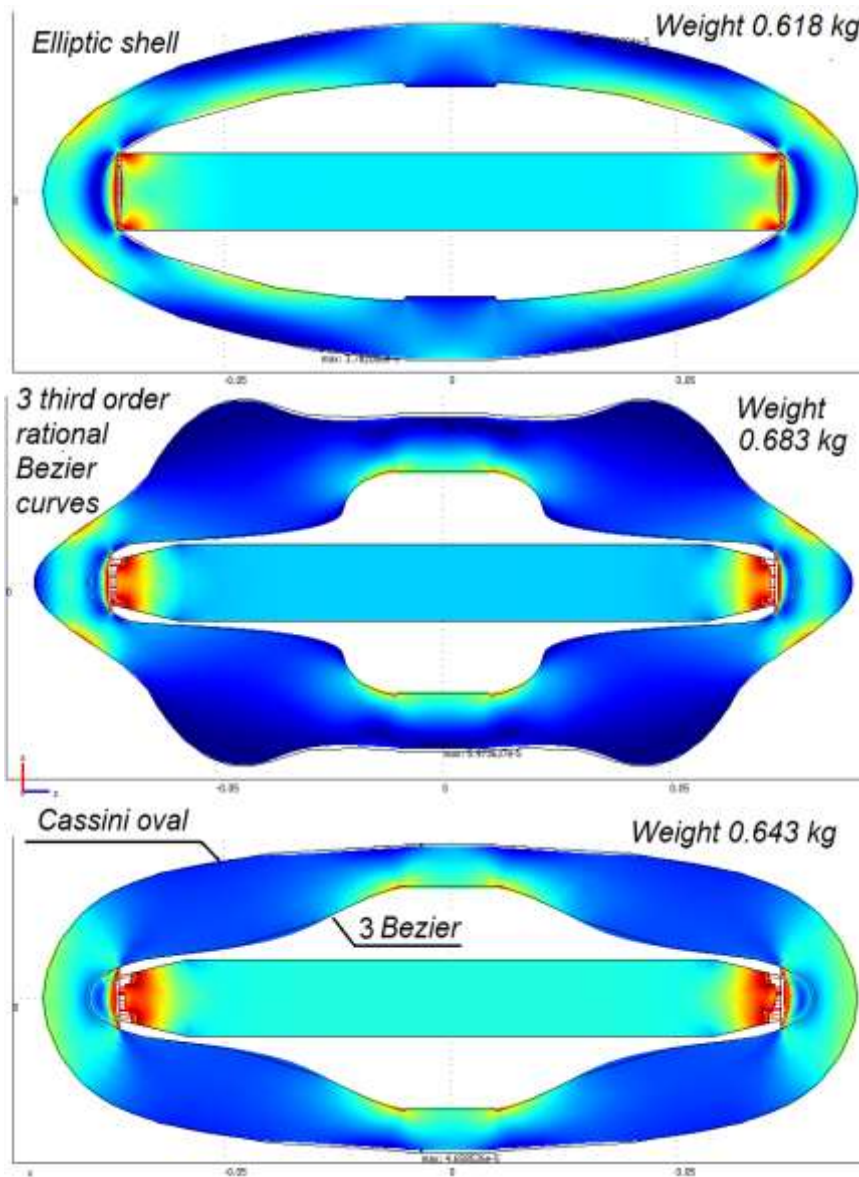


Figure 11. Compared actuators with different forms of shells.

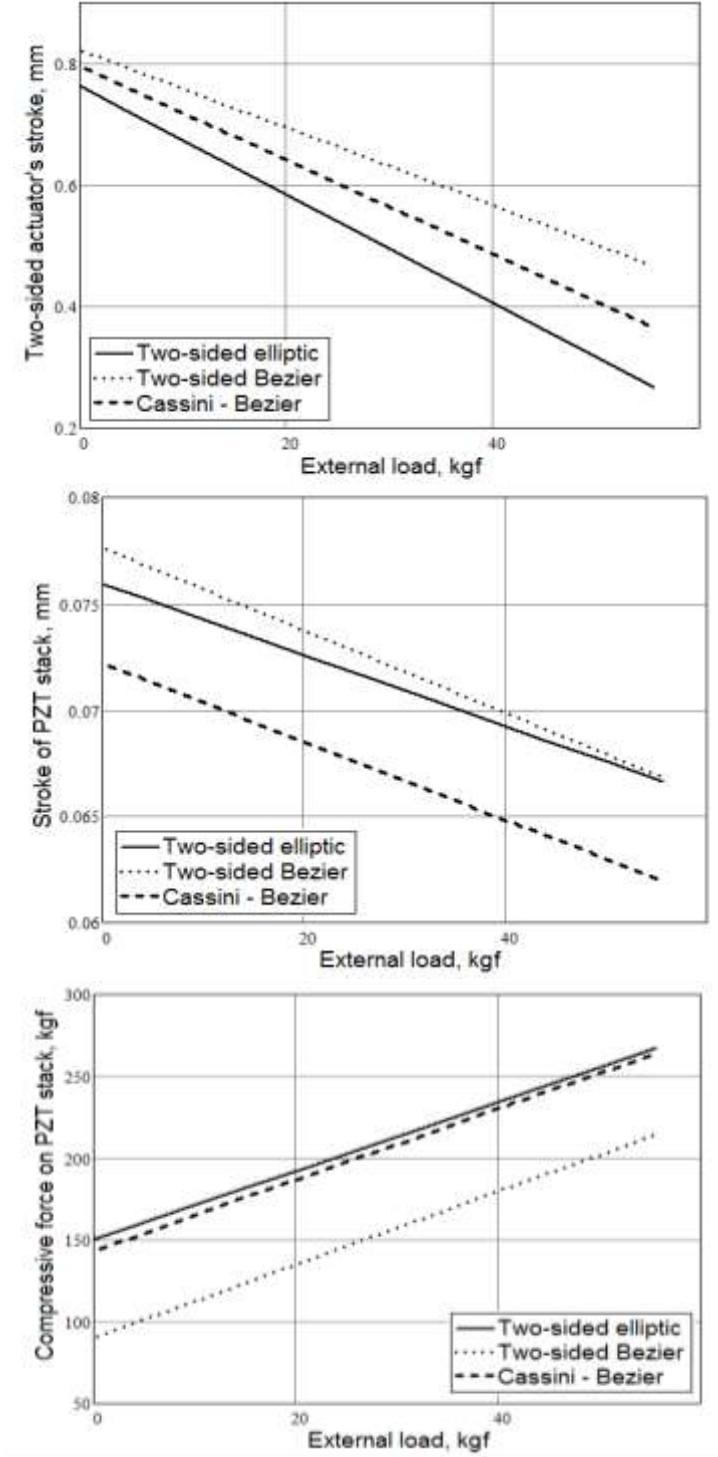


Figure 12. Performance parameters of three compared flextensional actuators with different shells.

4. RESULTS AND DISCUSSION

The developed optimization technique has been successfully applied to the different flextensional actuators with polymeric composite shell of the same outer dimensions $180 \times 70 \times 24 \text{ mm}^3$. Multilayered PZT stacks with dimensions $144 \times 24 \times 16 \text{ mm}^3$ were controlled by the same voltage (electric field 400 V/mm). All optimized actuators with improved parameters have a more complicated shape of the generatrix and a varied thickness along it. For the case when the shape of external generatrix is restricted by the smooth curves with no kinks (structural restrictions due to complexity of mould for the shell cure) all outer control point were fixed in positions appropriate to the Cassini oval [12] which is described by the equation:

$$r^4 - 2a^2r^2 \cos 2\theta = b^4 - a^4, \quad (10)$$

where the parameters a, b , which determine the shape of curve, are linked by the relationship $b/a > 1$, and spatial coordinates vary with the polar angle θ as $x = r \cos \theta$; $y = r \sin \theta$.

Three actuators with different shells have been compared: with elliptic, two-sided Bezier, and Cassini – Bezier generatrices (see Figure 11).

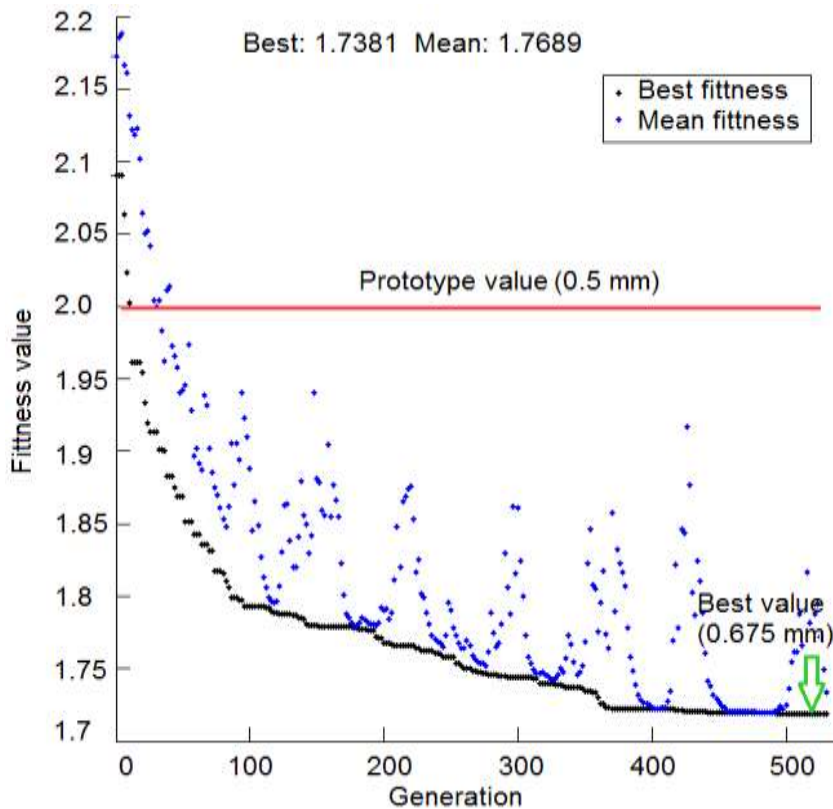


Figure 13. Diagram of the best fitness convergence.

The following plots (see Figure 12) shows comparative data of three investigated similar size actuators: with the elliptic shell and with shells optimized by the proposed method. These data demonstrates several best features of the optimized actuators. Actuator with two generatrices in the form of optimal Bezier curves is the best. At the load 40 kgf it provides a stroke half times greater than the actuator with a simple elliptical shell. In addition, at the same external load, it transmits a much smaller force on the piezoelectric stack that allows us to reduce its cross-section and hence the total mass of actuator. These advantages are crucial, since the power actuators are designed to move against the acting forces and should not change the mass balance of rotor blade.

Actuator with Cassini – Bezier shell has the intermediate parameters, but more preferable compared to simple elliptic shell.

The total computer time engaged by the process of optimization (about 500 epochs) was 5 – 6 hours on i5 computer. To illustrate character of optimization process the typical diagram of the fitness function convergence is presented in Figure 13. Here the solid red line shows the value of stroke for the actuator with elliptic shape. For all investigated structures the optimization using genetic algorithm and chosen fitness function ensures good repeatability and finite element mesh independence.

The developed method has been used to design the actuators for helicopter rotor control.

CONCLUSION

To provide an actively controlled flap for a helicopter rotor blade an optimization method for flextensional piezoelectric actuator has been developed and tested. This method is based on the representation of the shape of polymeric composite shell by the rational Bezier curves, whose parameters (coordinates and weights of the control points) are the design variables for the optimization algorithm. The optimization process is controlled by the genetic algorithm Toolbox MATLAB, which access a finite element model of device, calculates the objective functional (the stroke of actuator against given external load), and modifies design variables to achieve the optimum. The effectiveness of the proposed method is examined by comparative analysis of the prototype structure with elliptic shape of the composite shell. Our results prove the usefulness of the developed optimization methodology and its attractiveness not only for the aircraft applications but also in many areas of electromechanics, automatics and nanopositioning.

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Chapter 55

COMBINATION TREATMENT OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION AND INTENSIVE OCCUPATIONAL THERAPY: A NOVEL THERAPEUTIC APPROACH FOR UPPER LIMB HEMIPARESIS AFTER STROKE

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ABSTRACT

Recently, low-frequency repetitive transcranial magnetic stimulation (rTMS) applied to the non-lesional hemisphere has been reported to improve motor function of the paretic upper limb after stroke. On the other hand, some clinical studies have confirmed the beneficial effect of intensive occupational therapy (OT) such as constraint-induced movement therapy for upper limb hemiparesis after stroke. Both low-frequency rTMS over the non-lesional hemisphere and intensive OT can facilitate neural activity of the lesional hemisphere, which compensates for impaired motor function of the paretic upper limb. We postulated that combined application of low-frequency rTMS and intensive OT might accelerate the improvement of motor function of the paretic upper limb in post-stroke patients. Therefore, we newly developed a 15-day in-patient protocol of combination treatment of these two interventions for this patient population. In the protocol, each patient was scheduled to receive 22 sessions of combination treatment with 20-min low-frequency rTMS of 1 Hz to the non-lesional motor cortex followed by intensive OT over 15 consecutive days. The program of intensive OT consisted of 60-min one-to-one training and 60-min self-exercise. The results of the multi-center study including more than 200 patients showed that our proposed protocol is a safe and feasible intervention. In addition, the protocol improved significantly motor function of the affected upper limb in studied patients. Furthermore, it seems that concomitant introduction of daily levodopa administration and botulinum toxin type A injection can augment the efficacy of the protocol. It may be a promising strategy to apply more potent

modalities of TMS such as theta burst stimulation instead of conventional low-frequency rTMS, combined with intensive OT. In conclusion, our proposed combination treatment of low-frequency rTMS and intensive OT can be a novel therapeutic intervention for post-stroke patients with upper limb hemiparesis.

INTRODUCTION

In this chapter, we first discuss the therapeutic concept of repetitive transcranial magnetic stimulation (rTMS) combined with intensive occupational therapy (OT) for post-stroke patients with upper limb hemiparesis. In the second part, we describe our experience using the combination treatment of low-frequency rTMS and intensive OT in the same patient population. We also comment on future directions regarding this therapeutic modality in research and clinical practice.

THERAPEUTIC CONCEPT OF rTMS COMBINED WITH INTENSIVE OT FOR UPPER LIMB HEMIPARESIS AFTER STROKE

Low-Frequency rTMS As a Therapeutic Tool

Transcranial magnetic stimulation (TMS) is a painless, safe procedure involving non-invasive brain stimulation (Hallett, 2007). Applying TMS in a repetitive manner, i.e., repetitive TMS (rTMS), can modulate local cortical neuronal excitability with effects ranging from up-regulation (facilitation) to down-regulation (suppression) of neuronal activity, depending on the frequency of stimulation (Huerta and Volpe, 2009). High-frequency rTMS (≥ 5 Hz) facilitates local neural activity, whereas low-frequency stimulation (≤ 1 Hz) depresses such activity (Chen et al., 1997; Maeda, Keenan, Tormos, Topka, and Pascual-Leone, 2000; Muellbacher, Ziemann, Boroojerdi, and Hallett, 2000; Wu, Sommer, Tergau, and Paulus, 2000). In cases of impaired neurological function due to stroke, rTMS as a therapeutic intervention should be applied to activate the cortical areas that compensate for the function. Using neuroimaging studies, some researchers revealed that the perilesional areas in the lesional hemisphere play an important role in motor functional recovery of the affected upper limb after stroke (Cramer et al., 1997; Feydy et al., 2002; Marshall et al., 2000; Weiller, Ramsay, Wise, Friston, and Frackowial, 1993). Based on these findings, we hypothesized that neural activation of the perilesional areas can induce motor functional recovery of the paretic upper limb after stroke. Considering the bidirectional effects of rTMS, two therapeutic approaches to activate the perilesional areas have been suggested; the direct and indirect approaches.

In the direct approach, facilitatory high-frequency rTMS is applied over the lesional hemisphere to directly up-regulate neural excitability in the perilesional areas (Figure 1). On the other hand, in the indirect approach, inhibitory low-frequency rTMS is applied to the non-lesional hemisphere to suppress neural activity of the non-lesional hemisphere with subsequent suppression of interhemispheric inhibition towards the lesional hemisphere. Finally, the perilesional areas are disinhibited from the interhemispheric inhibition, resulting in up-regulation of the neural activity in these areas (Figure 2).

Whether the direct or indirect approach is more beneficial for upper limb hemiparesis after stroke remains to be clarified. Rossi, Hallett, Rossini, and Pascual-Leone (2009) have suggested that high-frequency rTMS poses a clear risk of seizure induction. In addition, low-frequency rTMS produces less site discomfort during stimulation than high-frequency rTMS.

Therefore, the indirect approach using low-frequency rTMS is safer and likely to be more widely applicable as a therapeutic intervention for upper limb hemiparesis after stroke compared with the direct approach using high-frequency rTMS. As shown in Table 1, the efficacy of low-frequency rTMS over the non-lesional hemisphere was established in many studies, and all showed significant improvement in motor function of the affected upper limb in post-stroke patients (Dafotakis et al., 2008; Fregni et al., 2006; Kirton et al., 2008; Mansur et al., 2005; Nowak et al., 2008; Takeuchi et al., 2005; Takeuchi et al., 2008).

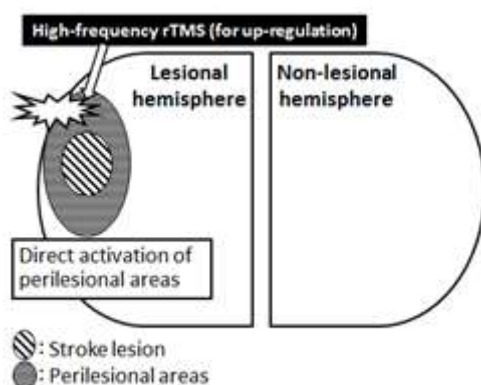


Figure 1. Direct approach. High-frequency rTMS is applied to the lesional hemisphere, to directly up-regulate neural excitability of the perilesional areas.

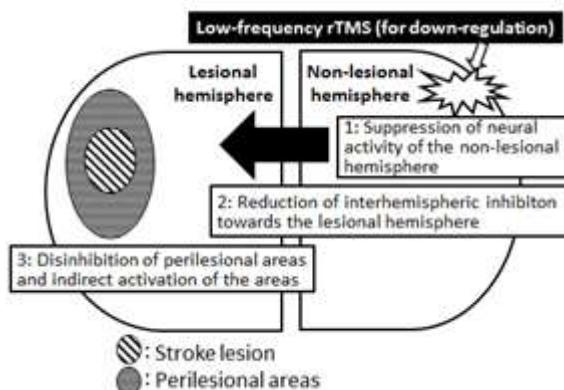


Figure 2. Indirect approach. Low-frequency rTMS is applied to the non-lesional hemisphere, resulting in the reduction of interhemispheric inhibition towards the lesional hemisphere. Subsequently, the perilesional areas are disinhibited from the interhemispheric inhibition and activated.

Notably, Fregni et al. (2006) indicated that improvement gained by daily application of low-frequency rTMS over a period of five consecutive days was maintained for up to 2 weeks after treatment cessation.

Based on these reports, we considered that low-frequency rTMS is a potentially useful therapeutic tool for upper limb hemiparesis after stroke.

Table 1. Reports of low-frequency rTMS over the non-lesional hemisphere for upper limb hemiparesis after stroke

Study	Intensity of stimulation (% of motor threshold)	Duration of one session (minutes)	No. of sessions (days)
Mansur et al. (2005)	100	10	1
Takeuchi et al. (2005)	90	25	1
Fregni et al. (2006)	100	25	5
Takeuchi et al. (2008)	90	25	1
Dafotakis et al. (2008)	100	10	1
Nowak et al. (2008)	100	10	1
Kirton et al. (2008)	100	20	8

* Pediatric stroke.

Combined Application of Low-Frequency rTMS and Intensive OT

Based on the beneficial effect of low-frequency rTMS over the non-lesional hemisphere on motor function of the paretic upper limb after stroke, combining this rTMS modality with another therapeutic intervention with proven beneficial effects for upper limb hemiparesis could enhance the treatment outcome. As the results of a meta-analysis, Langhorne, Coupar, and Pollock (2009) indicated that constraint-induced movement therapy (CIMT), a typical form of intensive OT, is the most effective rehabilitative intervention available. In addition, the results of the Extremity Constraint Induced Therapy Evaluation (EXCITE) trial of more than 200 post-stroke patients confirmed the significant benefit of CIMT for treating upper limb hemiparesis after stroke (Wolf et al., 2006). As an underlying mechanism of beneficial effects with intensive OT, it has been reported that intensive OT can facilitate neural activity of perilesional areas in the lesional hemisphere (Lewy, Nichols, Schmalbrock, Keller, and Chakeres, 2001; Wittenberg et al., 2003). We thus speculated that the combination treatment of low-frequency rTMS and intensive OT could produce greater improvement in motor function of the affected upper limb, through further facilitation of neural activity in the lesional hemisphere, compared with rTMS alone. Figure 3 illustrates our therapeutic concept using such therapeutic interventions.

As a pre-conditioning intervention, low-frequency rTMS is applied first to maximize brain plasticity in the lesional hemisphere. Following rTMS application, intensive OT was then provided to further facilitate neural activity in the perilesional areas, with consequent beneficial functional reorganization in the lesional hemisphere. In other words, we assumed that low-frequency rTMS over the non-lesional hemisphere conditions the compensating areas in the lesional hemisphere to be more responsive to the effects of intensive OT.

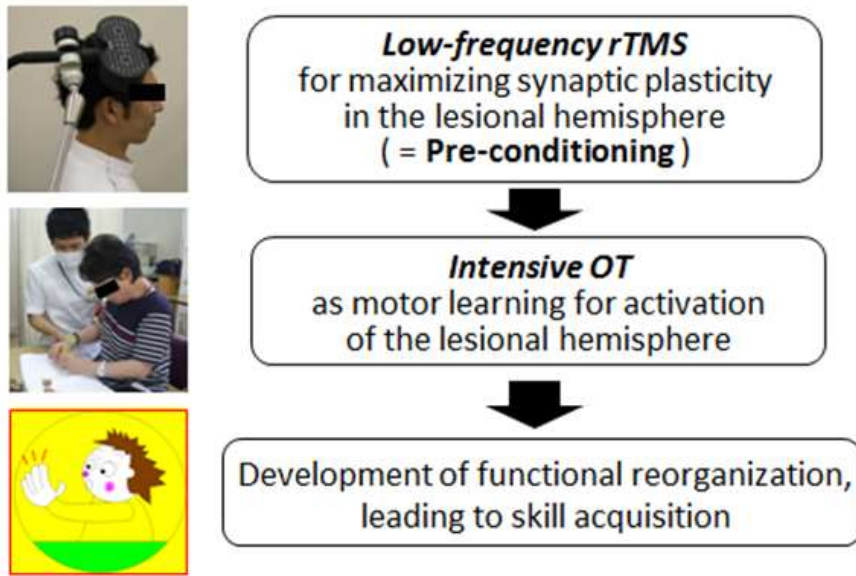


Figure 3. Therapeutic concept of our proposed combination treatment. Prior to intensive OT, low-frequency rTMS is applied as a pre-conditioning intervention for maximizing brain plasticity.

In-Patient Protocol of Combination Treatment

In 2008, we introduced the combination treatment of low-frequency rTMS and intensive OT as a 15-day protocol for post-stroke in-patients with upper limb hemiparesis at our university hospital (Kakuda et al., 2010). After confirming the protocol safety in a small number of patients at our university hospital, some other hospitals in Japan also started to provide the same protocol of combination treatment. Currently, eight hospitals in different parts of Japan (Jikei University Hospital, Jikei Daisan Hospital, Shimizu Hospital, Tokyo General Hospital, Nishi-Hiroshima Rehabilitation Hospital, Aizawa Hospital, Suwanomori Hospital, and Kimura Hospital) use our proposed protocol of the combination treatment as an in-patient intervention. All these hospitals have an at least 30-bed ward/section specially equipped for long-term rehabilitation of stroke patients, two or more board-certificated physicians, and at least five occupational therapists with expertise in stroke rehabilitation. Prior to the introduction of the protocol, physicians and therapists at each institution received training at our university hospital. All participating hospitals use the same inclusion criteria, therapeutic protocol, and clinical measures for assessment of the protocol efficacy.

Inclusion Criteria

The following are the currently used criteria in the above hospitals to determine the suitability of post-stroke patients for the treatment: 1) Brunnstrom stage for hand-fingers of 3-5 (ability, at least subjectively, to flex all the fingers of the affected upper limb in full range of motion); 2) age at intervention of 18-90 years; 3) more than 12 months between the onset of

stroke and treatment; 4) history of a single stroke only (no bilateral cerebrovascular lesion); 5) no cognitive impairment with a pretreatment Mini Mental State Examination score of more than 26; 6) clinical confirmation of the plateau state, representing no increase in the Fugl-Meyer Assessment (FMA) score assessed by an occupational therapist from the institution in the latest 3 months; 7) no active physical or mental illness requiring medical management; 8) no recent history of seizure (within one year preceding the intervention); 9) no documented epileptic discharge on pretreatment electroencephalogram; 10) no current use of antiepileptic medications for the prevention of seizure; and, 11) no pathological conditions known to be contraindications for rTMS, as listed in the guidelines of Wassermann (e.g., intracranial implants, cardiac pacemakers, pregnancy) (Wassermann, 1998).

Time Schedule of 15-Day Protocol

All patients were hospitalized in order to receive the 15-day protocol. During hospitalization, each subject received 22 treatment sessions, each comprising 20 minutes for application of low-frequency rTMS and 120 minutes of intensive OT (two sessions per day, except for the days of admission/discharge and Sundays) (Figure 4).

Application of Low-Frequency rTMS

At all hospitals, the rTMS was delivered using a 70-mm figure-8 coil and MagPro R30 stimulator (MagVenture Company, Farum, Denmark). In each 20-minute session, low-frequency rTMS of 1,200 pulses of 1 Hz was applied over the motor cortex of the non-lesional hemisphere, at the site that elicited the largest motor-evoked potentials (MEPs) in the first dorsal interosseous (FDI) muscle of the unaffected upper limb (confirmed by surface electromyographic recording).

	Thursday	Friday-Saturday	Sunday	Monday-Saturday	Sunday	Monday-Wednesday	Thursday
Morning	Admission	Low-frequency rTMS (20min)	No treatment	Low-frequency rTMS (20min)	No treatment	Low-frequency rTMS (20min)	Post-therapy evaluation
		One-to-one training (60min)		One-to-one training (60min)		One-to-one training (60min)	
		Self-exercise (60min)		Self-exercise (60min)		Self-exercise (60min)	
Afternoon	Pre-therapy evaluation	Low-frequency rTMS (20min)		Low-frequency rTMS (20min)		Low-frequency rTMS (20min)	Discharge
		One-to-one training (60min)		One-to-one training (60min)		One-to-one training (60min)	
		Self-exercise (60min)		Self-exercise (60min)		Self-exercise (60min)	

Figure 4. Time schedule of 15-day protocol of combination treatment. Except for days of admission/discharge and Sundays, patients were scheduled to receive two sessions of rTMS and intensive OT daily.

The intensity of the stimulation was set at 90% of motor threshold of the FDI muscle, which was defined as the lowest intensity of stimulation that could activate MEPs in the muscle.

For safety monitoring, a physician at each hospital briefly examined the patient before and after each rTMS session, paying attention to the possible development of adverse effects of rTMS (e.g., headache, nausea, convulsion), appearance of new neurological symptoms (e.g., motor disturbance of the unaffected limbs), or deterioration of upper limb hemiparesis.

Rehabilitative Program of Intensive OT

The program of intensive OT comprised two components: a 60-minute one-to-one training and a 60-minute self-exercise. The one-to-one training, which was provided by an occupational therapist, mainly involved shaping practices (e.g., reaching forward to move a cup from one place to another, writing letters and pictures using a pencil, wiping the surface of the table with a towel, picking up a hairbrush and combing hair) and repetitive task practices (e.g., clay squeezing and molding, pinching small coins, gripping a small ball). In both components of the protocol, patients were instructed to concentrate on use of the affected upper limb.

Although both of these two practices were always included at almost the same proportion of training time (usually 30 minutes for shaping practice and 30 minutes for repetitive task practice) in the one-to-one training program of each patient, the program was tailored by the therapist to suit the individual patient, based on motor function of the affected upper limb and lifestyle of the patients (e.g., occupation, interest, household work). Certain conventional approaches such as facilitation techniques and manual dexterity exercises were also sometimes included in the program. During the hospitalization, the program was modified following motor functional improvement of the affected upper limb.

All the one-to-one training tasks were generally supervised in a face-to-face fashion by an occupational therapist. Positive verbal guidance, encouragement, and feedback were frequently provided so as to achieve the best performance for the selected tasks. The therapists also provided “hands-on” facilitation of movement and inhibition of inappropriate muscle contraction, if necessary.

Following the one-to-one training session, self-exercise was administered in another quiet room without any supervision by the therapist (the room was under continuous video monitoring using built-in-camera for patient safety). Prior to each self-exercise, each patient received specific written instructions for tasks similar to those applied in the one-to-one training, generally involving 3-8 tasks per self-exercise session, with some rest breaks of a few minutes.

After each self-exercise session, the occupational therapists checked and reviewed performance of the tasks through an interview. Problem associated with the tasks were approached aggressively in the following one-to-one training session.

Clinical Evaluation of Upper Limb Motor Function

To determine the effect of the therapeutic protocol on motor function of the affected upper limb, FMA and Wolf Motor Function Test (WMFT) were applied on the days of admission and discharge, and four weeks after discharge whenever possible. The FMA includes 33 items for the evaluation of upper limb motor function (Gladstone, Danells, and Black, 2002; Platz et al., 2005). As each item is rated on a three-point ordinal scale (0=cannot perform, 1=can perform partially, 2=can perform fully), 66 points is the maximum possible score for motor performance of the upper limb. The WMFT includes 15 timed tasks for the evaluation of upper limb motor function, with the performance time of each timed task recorded (Morris, Uswatte, Crago, Cook, and Taub, 2001; Wolf et al., 2001). When the task is not completed within 120 seconds, the performance time of the task was recorded as 120 seconds. For statistical analysis, the mean value of WMFT performance time of 15 tasks was transformed to a natural logarithm as WMFT log performance time, to normalize the skewed distribution of the data, as applied in the analysis of data of the EXCITE trial (Wolf et al., 2006).

CLINICAL RESULTS

By the end of January 2011, 204 post-stroke patients had received the protocol at some of the above mentioned hospitals. In this section, we describe the clinical results of the protocol in these patients (Kakuda et al., 2012).

Clinical Features of Studied Patients

As shown in Table 2, the mean age of the patients at admission was 58.5 ± 13.4 years. The mean time between stroke onset and the intervention was 5.0 ± 4.5 years. Stroke was classified into intracerebral hemorrhage in 107 patients and cerebral infarction in 97 patients.

Safety and Feasibility of the Protocol

The 15-day treatment protocol was completed by all patients. No significant change in the vital signs was observed in any patients throughout the in-patient treatment. Furthermore, none of the patients developed new symptoms or noticed any deterioration of motor function in the upper limb during hospitalization. In 79 patients who received follow-up evaluation at four weeks after discharge, no new adverse symptoms or signs were recorded after the discharge.

Based on these observations, we concluded that the protocol was safe and feasible.

Treatment Outcome on Motor Function of the Affected Upper Limb

The significance of the median changes in FMA score and WMFT log performance time following the treatment was analyzed using the signed Wilcoxon's rank sum test for paired samples. All statistical analyses were performed using The Statistical Package for Social Sciences, v17.0 (SPSS Inc., Chicago, IL).

Based on the data from all 204 participating patients, the median FMA score increased significantly from 47 (36-54) [median (interquartile range)] points at admission to 51 (42-57) points at discharge (Figure 5). Similarly, the 15-day protocol significantly reduced the median WMFT log performance time from 3.23 (1.70-4.07) to 2.51 (1.36-3.86) (Figure 6).

Table 2. Demographic data of the studied patients

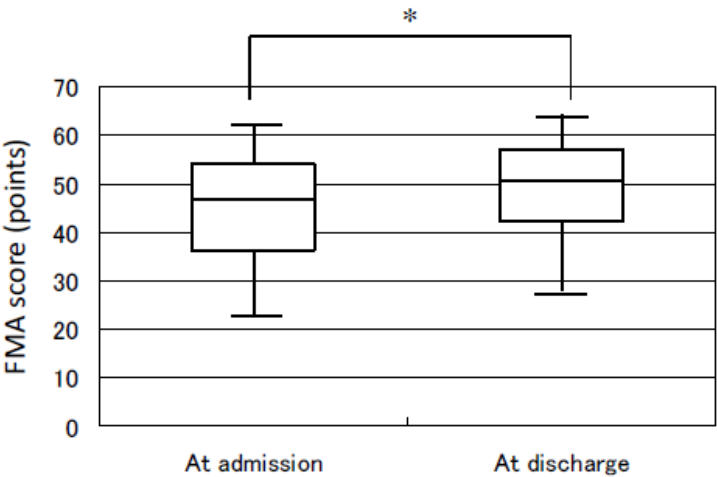
Age at admission, years	58.5 ± 13.4
Gender	
Females	73 (36)
Males	131 (64)
Time since stroke onset, years	5.0 ± 4.5
Subtype of stroke	
Intracerebral hemorrhage	107 (53)
Cerebral cortical infarction	27 (13)
Lacunar infarction	70 (34)
Side of upper limb hemiparesis	
Dominant hand	124 (61)
Non-dominant hand	80 (39)

Values are numbers (%) or mean ± standard deviation.

In 79 patients who were also evaluated at 4 weeks after discharge, both the increased FMA score and decreased WMFT log performance time remained significant compared to the respective values at admission [FMA: 48 (range, 34-53) points at admission, 51 (38-57) points at discharge, 50 (34-56) points at 4 weeks after discharge; WMFT log performance time: 2.81 (1.42-4.08) at admission, 2.20 (1.25-3.78) at discharge, 2.01 (1.31-3.94) at 4 weeks after discharge]. These results suggested that our proposed 15-day protocol improves motor function of the paretic upper limb and that the improvement is maintained for at least 4 weeks after cessation of the protocol.

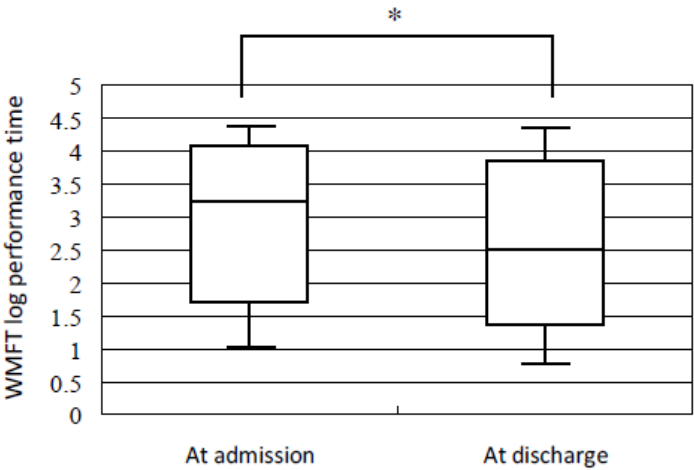
Baseline Features Affecting Treatment Outcome

In order to identify the factors that correlated significantly with the outcome (i.e., significant change in FMA score and WMFT log performance time), linear regression analysis was performed using the following five baseline characteristics: age, gender, time since stroke onset, subtype of stroke (intracerebral hemorrhage/cerebral cortical infarction/lacunar infarction), and side of upper limb hemiparesis (dominant hand/non-dominant hand).



*p<0.001.

Figure 5. Changes in FMA score in all studied patients. Bars represent the median, and 5th, 25th, 75th, and 95th percentiles.



*p<0.001.

Figure 6. Changes in WMFT log performance time in all studied patients. Bars represent the median, and 5th, 25th, 75th, and 95th percentiles.

When FMA score or WMFT log performance time was selected as the dependent variable, no significant relationship with any of the five parameters was found, indicating that no clinical factor tested thus far definitely influences the functional response to the protocol.

FUTURE DIRECTIONS IN RESEARCH AND CLINICAL PRACTICE

The present study suggested that the combination treatment of low-frequency rTMS and intensive OT safely improves motor function of the paretic upper limb in post-stroke patients.

However, the described protocol was not necessarily satisfactory for all the patients treated, and further improvements in efficacy and extent of benefit of the protocol are needed to ensure that more patients receive the therapeutic effects.

We have therefore proposed further modifications to the protocol for future clinical use in improving motor functional recovery, and initiated pilot studies to assess the safety and effect of these modified protocols. The ideas involve levodopa administration, botulinum toxin injection, and the application of more potent TMS modalities.

CONCOMITANT ADMINISTRATION OF LEVODOPA

Some neurotransmitters modulate brain plasticity after brain damage. In particular, noradrenaline in the brain seems important for facilitating motor functional recovery in the brain following damage (Boyeson and Feeney, 1989). However, such a molecule can have severe adverse effects on various systems such as the cardiovascular system.

On the other hand, administration of levodopa, which is partly metabolized to noradrenaline in the brain, is considered safe and feasible to increase the brain concentration of noradrenaline (Nutt and Fellman, 1984). A recent randomized, prospective, double-blinded study of post-stroke patients found that levodopa administration of 100 mg daily for three weeks in addition to physiotherapy could enhance motor recovery in post-stroke hemiparetic patients (Scheidtmann, Fries, Muller, and Koenig, 2001).

Furthermore, none of these patients showed severe adverse effects associated with levodopa, indicating its safety and feasibility in such a patient population. Therefore, we hypothesized that concomitant administration of levodopa could further facilitate the beneficial plastic changes in the brain produced by our combination treatment.

In other words, levodopa administration could condition the brain to be more responsive to the combination treatment of rTMS and intensive OT. Figure 7 illustrates our novel protocol featuring levodopa administration (Kakuda et al., 2011b). Patients were scheduled to receive a daily oral administration of 100 mg levodopa for one week prior to admission. If the administration was tolerated for one week, the full 15-day in-patient protocol was commenced, along with levodopa administration. The administration was continued until four weeks after the discharge. We recommend an aggressive administration of levodopa concomitantly with our combination treatment, as such a regimen is easy to clinically administer and is not associated with a high risk of adverse effects.

PRE-TREATMENT INJECTION OF BOTULINUM TOXIN TYPE A

For some patients with marked upper limb spasticity as well as upper limb hemiparesis, it seemed that our current protocol of combination treatment did not work well (Kakuda et al., 2011c).

For such patients, a focal injection of botulinum toxin type A (BTXA) prior to the combination treatment is recommended. In recent years, BTXA has been used to control focal spastic hypertonia in patients with upper motor neuron disturbances. Focal injection of BTXA inhibits the release of acetylcholine at the neuromuscular junction and reduces spasticity of

the injected muscle (Brashear et al., 2002; Richardson et al., 2000). Since the BTXA is most effective 4-8 weeks after the injection, the focal injection should be performed at the clinic 4 weeks prior to the in-patient combination treatment (Kaji et al., 2010).

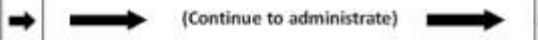
Outpatient treatment		In-patient combination treatment				Outpatient treatment
One week before admission		On the day of admission	2-14 days of admission (except for Sundays)	On the day of discharge		Four weeks after discharge
Start of levodopa administration (orally 100mg/day)						
		AM	Admission	Low-frequency rTMS (20 min)	Post-therapy evaluation and instruction for self-training	Follow-up evaluation
				One-on-one training (60 min)		
				Self-training (60 min)		
		PM	Pre-therapy evaluation	Low-frequency rTMS (20 min)	Discharge	
				One-on-one training (60 min)		
				Self-training (60 min)		

Figure 7. Novel protocol featuring levodopa administration. Patients are requested to receive daily levodopa administration concomitantly with the combination treatment of rTMS and intensive OT.

The selected dose was based on the severity of spasticity and the volume of the target muscles. Based on previous reports, the selected target muscles were in the following order: biceps brachii muscle, flexor carpi radialis muscle, flexor carpi ulnaris muscle, flexor digitorum superficialis muscle, flexor digitorum profundus muscles, flexor pollicis longus muscle, and adductor pollicis muscle (Brin, 1997). The maximum total dose of injected BTXA was 240 units. The pilot study revealed significant improvement in motor function of the affected upper limb, in addition to a significant reduction in spasticity following the intervention. It is possible that the beneficial effects of our proposed protocol will be reduced with the disappearance of the toxin effect; however, the anti-spastic effect of intramuscular BTXA could be successfully maintained by repeated injections 12-20 weeks apart (Bakheit et al., 2004; Elovic et al., 2008). It is expected that such a procedure can maintain the improved motor function in patients who received our combined therapeutic protocol over a long period of time (beyond the expiration of the first injection of BTXA) when BTXA is injected at regular intervals.

APPLICATION OF MORE POTENT TMS MODALITIES FOR NEUROMODULATION

Recently, certain TMS modalities have been reported to be more powerful than conventional rTMS in modulating brain plasticity, including theta burst stimulation (TBS), rTMS with priming stimulation (primed rTMS), paired-pulse stimulation, and quadro-pulse stimulation (Cardenas-Morales, Nowak, Kammer, Wolf, and Schonfeldt-Lecuona, 2010;

Carey, Anderson, Gillick, Whitford, and Pascual-Leone, 2010; Hamada et al., 2007; Huang, Edwards, Rounis, Bhatia, and Rothwell, 2005; Iyer, Schleper, and Wassermann, 2003; Thickbroom, Byrnes, Edwards, and Mastaglia, 2006). Among these modalities, we suggest the application of TBS and primed rTMS instead of the conventional rTMS used in our current protocol, since the safety of both these other modalities have been confirmed and some reports describing their clinical use are already published. The TBS comprises three pulses of 50 Hz delivered every 200 ms, simulating a theta-like rhythm. The pattern of delivery of TBS (continuous TMS versus intermittent TBS) is crucial in determining whether the excitability of the motor cortex is increased (intermittent TBS) or decreased (continuous TBS), with both deliveries inducing after-effects that last longer than any other known rTMS protocol (Cardenas-Morales et al., 2010; Huang et al., 2005). Other advantages of TBS include the shorter application time and the lower stimulation intensity needed to modulate cortical excitability, compared to conventional rTMS modalities. Therefore, a promising strategy for the application could be continuous TBS over the non-lesional hemisphere instead of the conventional low-frequency rTMS, combined with intensive OT for upper limb hemiparesis after stroke. Confirming the usefulness of primed rTMS, Iyer, Schleper, and Wassermann (2003) demonstrated that immediately preceding 1-Hz rTMS with “priming” trains of 6-Hz rTMS caused significantly greater depression of cortical excitability in the stimulated hemisphere than without such priming. This TMS modality also can be clinically applied as an intervention for suppressing neural activity of the non-lesional hemisphere. In our novel protocol featuring this 6-Hz primed low-frequency rTMS, a session of priming stimulation comprising 10 minutes of intermittent 5-Hz rTMS given in 5-s trains with 25-s intervals between trains (Kakuda et al., 2011a). Immediately following the priming stimulation, low-frequency pulses of 1 Hz were applied for 20 min. The result of a pilot study of combined 6-Hz primed low-frequency rTMS and intensive OT showed the beneficial effect of this protocol for upper limb hemiparesis after stroke.

CONCLUSION

Low-frequency rTMS applied together with intensive OT significantly improved motor function of the paretic upper limb after stroke, and such improvement was not associated with any adverse events. Although its efficacy needs to be confirmed in a randomized controlled trial with a control group, we are confident that the protocol is therapeutically beneficial for post-stroke patients with upper limb hemiparesis. We expect that the efficacy of the protocol could be improved with the introduction of concomitant use of pharmacological approaches and application of more potent TMS modalities.

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Chapter 56

EVIDENCE FOR C-REACTIVE PROTEIN AS A PROGNOSTIC INDICATOR FOR ISCHAEMIC STROKE RISK

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ABSTRACT

Background: C-reactive protein (CRP) is a blood marker of inflammation and a hallmark of the acute-phase response. Increased CRP is a known predictor of vascular events in asymptomatic individuals and stroke patients. We assessed CRP levels in addition to traditional risk factors in a cohort of transient ischaemic attack (TIA) patients to examine the relationship of these parameters to the occurrence of ischaemic stroke.

Methods: This is a prospective, longitudinal clinical evaluation of the efficacy of CRP as a prognostic indicator. CRP levels were measured in asymptomatic individuals and in TIA patients. 1024 asymptomatic individuals plus the 208 TIA patients (who met all the inclusion and data-collection criteria) were included in the ROC and AUC analysis, making a total of 1232 samples. A clinical risk score was determined in TIA patients using the ABCD² score. Two models were evaluated: ABCD²-only Model, and ABCD²+CRP Model. The primary outcome was an incident ischaemic stroke.

Results: Within 3.7 years ischaemic strokes occurred in 48/208 patients. The Cox proportional hazards models, after adjustments for conventional risk factors, identified CRP levels ≥ 3 mg/L and ABCD² scores ≥ 4 as independent predictors of stroke. The corresponding AUCs were 0.622 and 0.691, based on ABCD²-only Model and ABCD²+CRP Model, respectively; this represented a statistically significant difference ($p = 0.028$). The absolute Integrated Discrimination Improvement (IDI) was 0.0438 ($p < 0.001$), and the relative IDI was 0.8926. The net benefit (NET) became significant from a predicted probability $\geq 20\%$ and was 0.080 when based on ABCD²-only Model and 0.097 when based on ABCD²+CRP Model.

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Conclusions: Routine CRP measurements in the acute phase might be a useful tool for identifying TIA patients who are at a higher risk of ischaemic stroke.

Keywords: cardiovascular diagnostic technique, transient ischaemic attack, blood proteins, stroke

INTRODUCTION

Biomarkers, such as C-reactive protein (CRP), which is a peripheral marker of inflammation, might represent a mediator of vascular disease [1]. CRP plays an important role in the development of cardiovascular disease [2, 3]. There is growing evidence that CRP is also a marker of generalized atherosclerosis [4]. Several papers that evaluated its role in ischaemic disease have been published in the past 10 years [5-8]. CRP levels have been measured in patients affected by stroke to facilitate the understanding of its role as a predictor of further cardio- and/or cerebro-vascular events [3-7].

The JUPITER trial shows that the use of rosuvastatin in patients with high CRP level has a significant impact both in reducing the CRP level and in lowering future vascular events [9]. The role of CRP as a marker of ischaemic stroke has been less extensively studied in comparison to coronary artery disease [1-3, 8].

According to published guidelines there is not sufficient evidence to recommend measurement of CRP in the routine evaluation of cerebrovascular disease in primary prevention [8]. In secondary prevention of stroke elevated CRP adds to existing prognostic markers, but it remains to be established whether specific therapeutic options can be derived from this [8].

There have been few studies that evaluated biomarkers exclusively in patients with transient ischaemic attacks (TIA), limited data have been published, and consensus on the significance of CRP levels in TIA patients remained elusive [10, 11]. The risk of stroke after a TIA can be predicted by scores incorporating age, blood pressure, clinical features, duration, and diabetes (ABCD²-score).

The accent on TIA is due to the early elevated risk of stroke (estimates suggest that approximately 20% of strokes are preceded by TIA) [12, 13]. The ABCD system was designed to predict early risk of stroke after TIA [14]. Current international guidelines have adopted this system in the risk stratification of TIA patients and recommend immediate hospitalisation of patients with an ABCD² score ≥ 3 [15] or an ABCD² score ≥ 4 or with crescendo TIA within the previous 7 days [16]. This score have the advantage of using easily assessable clinical variables.

Despite the development of clinical risk prediction scores, such as the ABCD² score, risk stratification remains imperfect. Doctors in the emergency setting need to rapidly and reliably obtain data for each patient for the development of individualised patient risk profiles and the implementation of clinical management plans tailored to each patient. With these considerations, we designed a study to test the hypothesis that a refinement of the ABCD² score by adding a biomarker (CRP) increases the predictive value of the ABCD² score.

METHODS

Two models were evaluated: ABCD²-only Model, and ABCD²+CRP Model. ABCD²-only Model was designed on the ABCD² prognostic score, and the score was calculated on the basis of the following: age (≥ 60 years, 1 point); blood pressure at presentation (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, 1 point); clinical features (unilateral weakness, 2 points; speech disturbance without weakness 1 point; and other symptoms, 0 points); duration of symptoms (≥ 60 minutes, 2 points; 10 to 59 minutes, 1 point; and < 10 minutes, 0 points); and presence of diabetes (1 point). The ABCD² scores were categorised into low (0 to 3) and high (≥ 4) risk. The duration of symptoms was determined using the last known time point when the patient was in normal condition as the reference point. When multiple TIAs occurred during the study period, the first event was used for calculation of the ABCD² risk score. The ABCD²+CRP Model was designed by adding the CRP value to the ABCD² score.

Subjects and Inclusion Criteria

This study included an analysis of the Cerebrovascular Aosta Registry (CARE) data, inclusion criteria, data collection and follow-up methods were previously described [17]. Baseline measurements were obtained between January 1st, 2004 and December 31st, 2008, and consisted of data related to the event, as well as clinical features that included gender, age, blood pressure, and visits to the Emergency Ward (EW) or to a Specialist Centre (SC); data were collected using a standardized case report form. A TIA was defined as a brief episode of neurological dysfunction that was caused by a focal disturbance due to brain or retinal ischaemia that lasted for less than 24 hours without evidence of infarction [18]. Stroke was defined as “rapidly developing focal (or global) clinical signs of cerebral dysfunction that lasted more than 24 hours with no apparent cause other than a vascular origin” [19]. The following variables were systematically assessed: demographic variables, medical history, physical examination, routine blood biochemistry, early cerebral CT scan and/or magnetic resonance imaging (MRI), carotid ultrasonography, electrocardiogram (ECG), Holter ECG, echocardiography (if indicated), and stroke preventive treatments. Patients with transient dizziness, isolated vertigo, epilepsy, acute confusional state, isolated diplopia, generalised weakness, and syncope were excluded. For the current study, we selected all patients with a final diagnosis of TIA, based on the consensus opinion of two stroke neurologists. Inclusion criteria included a TIA with a CRP measurement within 48 hours after symptom onset. Cases that involved chronic inflammatory disease, cancer or infection before or after the TIA were excluded. All possible predictor variables, such as hypertension, history of myocardial infarction (IHD), atrial fibrillation (AF), diabetes, smoking, and body mass index (BMI), were recorded (Table 1). Patients were treated according to the current guidelines. This included life-style counselling, antihypertensive drugs, antithrombotic agents, statins, as well as specific interventions tailored to the etiology. If carotid stenosis was $\geq 70\%$, the patient was a candidate for carotid intervention within 2 weeks. One thousand twenty-four asymptomatic individuals plus the 208 patients (who met all the inclusion and data-collection criteria) were included in the ROC and AUC analysis, making a total of 1232 samples.

Table 1. Demographic data, cardiovascular risk factors and baseline laboratory parameters according to the CRP cut-off

	CRP <3 mg/l	CRP ≥3 mg/l	P values
Patients, n	79	129	
Mean age, years	69.7 (66.5-72.8)	73.7 (71.5-75.9)	0.018
Gender (M/F), n	36/43	64/65	NS
Mean glucose, mg/dl	100.9 (97.3-104.5)	115.5 (107.1-123.9)	0.005
Mean cholesterol (LDL), mg/dl	120.5 (112.3-128.6)	125.7 (117.9-133.5)	NS
Mean BMI	24.6 (23.9-25.2)	26 (25.3-26.7)	0.002
Risk factors (n,%)			
Hypertension*	69 (87.3%)	117 (90.7%)	NS
Diabetes*	9 (11.4%)	35 (27.1%)	0.007
AF	15 (19%)	25 (19.4%)	NS
>2 risk factors	14 (17.7%)	40 (31%)	0.034
Clinical features (n,%)			
Unilateral weakness	22 (27.8%)	46 (35.7%)	NS
Speech disturbance without weakness	43 (54.4%)	68 (52.7%)	NS
Other	14 (17.7%)	15 (11.6%)	NS
Duration of symptoms (n,%)			
<10 min	13 (16.5%)	12 (9.3%)	NS
10–59 min	51 (64.6%)	84 (65.1%)	NS
≥60 min	15 (19%)	33 (25.6%)	NS
ABCD2 at admission (n,%)			
Score 0–3	35 (44.3%)	30 (23.3%)	0.001
Score 4–7	44 (55.7%)	99 (76.7%)	0.001
Symptomatic internal carotid stenosis ≥50% or occlusion (n,%)			
Present	9 (11.4%)	26 (20.2%)	NS
Absent	70 (88.6%)	103 (79.8%)	NS
Secondary prevention therapy at discharge (n,%)			
BP-lowering	55 (69.6%)	97 (75.2%)	NS
Antithrombotic drugs	67 (84.8%)	103 (79.8%)	NS
Warfarin	11 (13.9%)	20 (15.5%)	NS
Statins	13 (16.5%)	21 (16.3%)	NS
Carotid endarterectomy	2 (2.5%)	7 (5.4%)	NS

*systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg, or current use of antihypertensive medications

• if fasting glucose ≥ 126 mg/dL or current use of blood sugar-lowering drugs.

BLOOD SAMPLING AND DETERMINATION OF CRP LEVELS

Blood samples were drawn within 48 hours after symptom onset and were blindly coded. In the laboratory of our hospital, a Roche automated Modular P analyser was used to measure CRP concentrations, according to the CRPLX test. A concentration ≥ 5 mg/L was defined as pathologically increased according to the reference values of our laboratory. CRP measurements were performed by an independent observer who was blinded to the clinical

characteristics of the patients. Control blood samples were obtained from an age-matched population without any history of TIA or stroke, but with risk factors that were similar to the analysed patients (1024 asymptomatic individuals were recruited from a project on stroke prevention, the PrATO, which was simultaneously ongoing in the Aosta Valley territory).

Carotid Ultrasonography

All patients underwent longitudinal 2-dimensional ultrasound examinations of the carotid arteries to evaluate the presence of plaques and to quantify the degree of stenosis. The examinations were performed by a vascular neurologist with an ESAOTE MyLab 70 XVision (Esaote, Genoa, Italy). The internal and common carotid arteries were classified as follows: 'normal', '< 50% stenosis', '50-69% stenosis', '≥ 70% stenosis but less than near occlusion', 'near occlusion' or 'occlusion' [20]. Stenoses that were ≥ 50% were considered symptomatic when they were ipsilateral to the ischaemic field. TIA patients were classified into 2 groups: a) no atherosclerosis or '< 50% stenosis' and b) from '≥ 50 stenosis' to 'occlusion' Patient Follow-up and Outcome Measurements. An independent neurologist followed up with each patient using a questionnaire that investigated the occurrence of new cerebrovascular events; complete data collection was assured by the analysis of the CARE stroke registry. The primary end point was an ischaemic stroke. Outcome was analysed until June 30st, 2011.

Statistical Analysis

The ROC curves were plotted for two models: Model ABCD²-only and Model ABCD²+CRP. If the model predicted and individual would have an ischaemic event within 3.7 years, but did not, it was considered a false-positive. We evaluated the improvement in model performance introduced by the inclusion of CRP value. The increase in the area under the receiver operating characteristic curves (AUC) was evaluated and tested for significance using the test proposed by DeLong et al. [21]. The usefulness of the new marker (CRP) was evaluated by the prognostic model described by Pencina et al. [22] and Vickers et al. [23]. The plasma concentration of CRP followed a log-normal distribution, and all CRP data were log-transformed for analysis.

Data are presented as the mean or the median, with the corresponding 95% CI or the interquartile range, as appropriate. We used the χ^2 test to compare the differences in the proportions of categorical variables, the Student's T test for continuous normally distributed variables (age, serum cholesterol) and the Mann-Whitney test for continuous non-normally distributed variables. P-values ≤ 0.05 were considered to be statistically significant for each test. We performed a univariate analysis of cardiovascular risk factors and recognised prognostic indicators of stroke, including the CRP levels, using a Cox proportional hazard regression model. Multivariate Cox regression models were performed to identify independent predictors of ischaemic stroke.

Ethics

The Medical Ethics Committee of Regional Hospital, Aosta Valley approved the study. Informed consent was obtained from all patients.

RESULTS

A total of 411 patients with a diagnosis of TIA between January 1st, 2004 and December 31st, 2008, were included in CARE registry. However, 203 patients did not fulfil the inclusion criteria for the present study: 76 did not have a CRP level measurement within 48 hours of TIA onset; 65 patients had a previous stroke; 27 patients had a history of recent infection; 15 patients had a history of cancer, 10 patients had a history of chronic inflammatory disease; and in 10 patients, data on the TIA duration were missing.

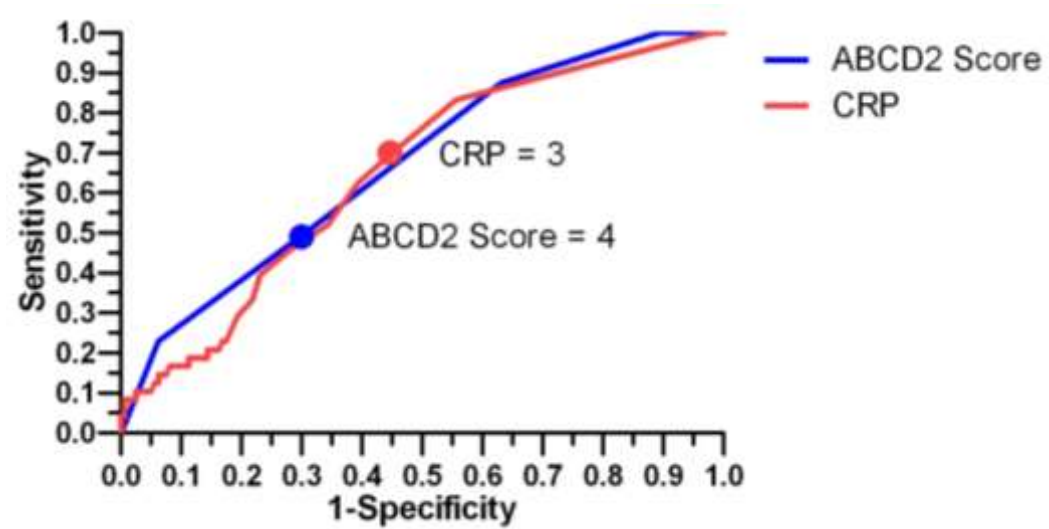


Figure 1. Receiver operating characteristic curves for the ABCD score and CRP level

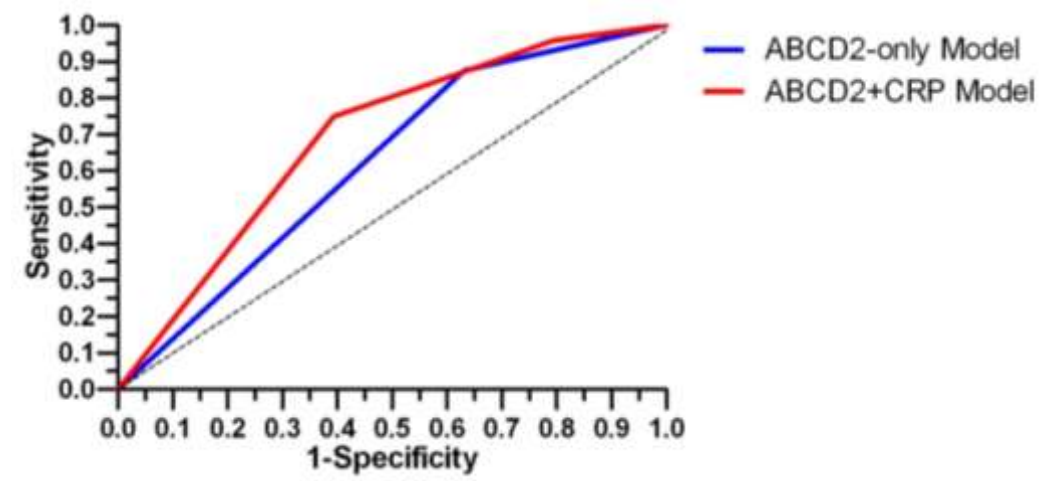


Figure 2 A. Area under the receiver operating characteristic curve (AUC) for two different predictive models.

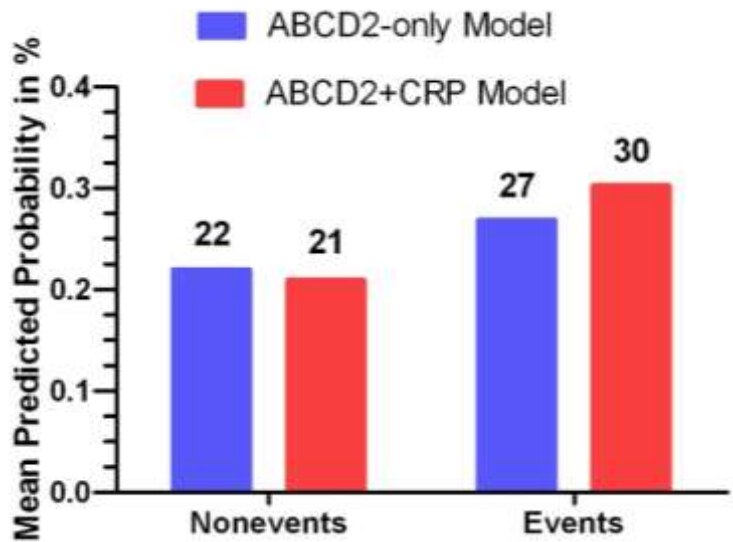


Figure 2 B. Mean Predicted Probability among TIA patients who experienced an incident ischaemic stroke and those who did not, based on ABCD2-only Model and ABCD2+CRP Model.

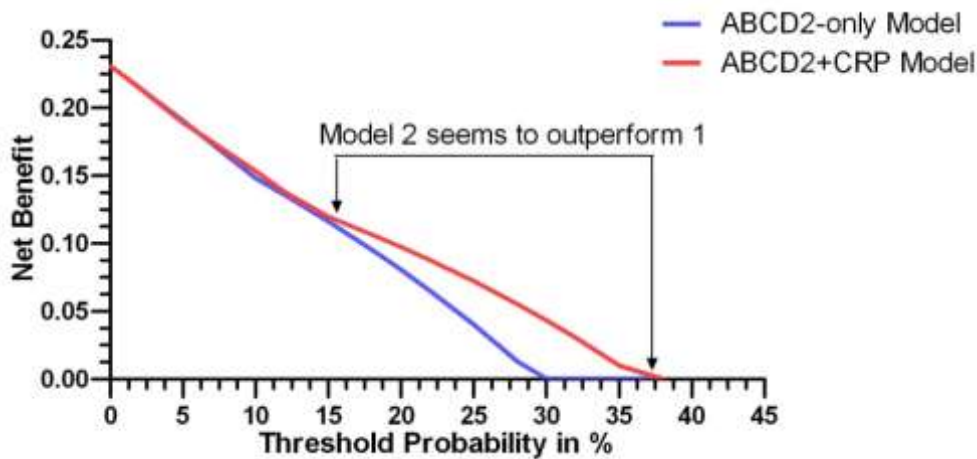


Figure 2 C. Graphical comparison between ABCD2-only Model and ABCD2+CRP Model, based on a ‘net’ benefit.

Therefore, the analysis for the present study was performed for the remaining 208 patients. Patients were evaluated at the EW (85%) and at the SC (15%). The median time between the onset of symptoms was 2.55 hours (95% CI 2 to 3.1). All patients underwent brain CT scan at a median time of 4.0 hours (95% CI 3.5 to 4.5) after symptoms onset. Of the 208 patients, 76 underwent a cerebral MRI during the acute phase, 38 patients in the CRP < 3 mg/L group (diffusion-weighted imaging (DWI) was positive in 4 patients) and 38 patients in the CRP ≥ 3 ml/L group (DWI was positive in 5 patients).

The difference between the number of DWI positive patients in the two subgroups was not statistically significant ($p = 0.68$). The median CRP levels was 3 mg/L (interquartile range

2 to 7). None of these patients had a recurrence before CRP measurement. The analysis of the ROC curves revealed that a CRP level of 3 mg/L was the best cut-off point for predicting the occurrence of end-point events and optimising of the sensitivity-specificity ratio (sensitivity = 0.70; specificity = 0.54; AUC = 0.65; 95% CI, 0.58 to 0.71; $p = 0.0004$); and ABCD score = 4 (sensitivity = 0.50; specificity = 0.69; AUC = 0.67; 95% CI, 0.60 to 0.73; $p < 0.0001$) (Figure 1). Accordingly, patients were divided into two subgroups: CRP level < 3 mg/L or CRP level ≥ 3 mg/L.

Demographic data, cardiovascular risk factors, baseline laboratory parameters, characteristics of the TIA patients, and therapy at discharge according to the CRP cut-off are reported in Table 1. The CRP ≥ 3 mg/L group was significantly older than the other group ($p < 0.018$), and the patients were more frequently diabetic. For secondary prevention at discharge, of the 208 patients, 170 (82%) were treated with antithrombotic medication; 31 patients (15%) were treated with warfarin (according to the international normalised ratio range between 2.5 and 3.5); and 7 patients (3%) did not receive any secondary prevention treatment. At discharge, a blood pressure-lowering medication was administered to 152 (73%) patients, lipid-lowering treatment (dose range between 10 and 40 mg/die) was administered to 34 (16%) patients and anti-diabetic medication was administered to 27 (13%) patients. No statistically significant difference for ischaemic stroke was found at the 28-days and 1-year follow-up based on the comparison of all of the patients ($n = 411$) included in CARE registry vs. the subgroup study analysis ($n = 208$): 8% vs. 11% ($p = 0.2$) and 15% vs. 18% ($p = 0.3$), respectively. During the 3.7 years of follow-up, 48 patients experienced an ischaemic stroke. The hazard ratios for ABCD² ≥ 4 and CRP ≥ 3 based on the Cox regression model was statistically significant for CRP (HR = 2.95, $p = 0.005$) (Table 2). The ROC curves for the ABCD²-only Model and ABCD²+CRP Model are presented in Figure 2A. AIC (Akaike Information Criterion) decreased from 486 to 479 on addition of CRP.

Table 2. Cox regression coefficients based on ABCD²-only Model and ABCD²+CRP Model of stroke events

Prognostic variables	Parameter estimate	Standard error	p values	Hazard Ratio	95% CI
Model ABCD ² -only (significance level =0.0004)					
ABCD ² score ≥ 4	1,32	0,44	0,0024	3,76	1,60 - 8,81
Model ABCD ² +CRP (significance level <0.0001)					
ABCD ² score ≥ 4	1,13	0,44	0,0101	3,10	1,32 - 7,33
CRP ≥ 3 mg/l	1,08	0,39	0,0056	2,96	1,38 - 6,34

The corresponding AUCs were 0.622 and 0.691, based on ABCD²-only Model and ABCD²+CRP Model, respectively, with a statistically significant difference ($p=0.028$), that showed an increase of 11.1%. The odds ratios were 3.34 (CI 95% 1.31 to 8.50, $p = 0.01$) and 3.35 (CI 95% 1.45 to 7.74, $p = 0.004$). for ABCD²-only Model and for ABCD²+CRP Model, respectively. Figure 2B illustrates the mean predicted probability of an ischaemic stroke based on ABCD²-only Model and ABCD²+CRP Model in those subjects who experienced an

ischaemic stroke and in those who did not have. The absolute Integrated Discrimination Improvement (IDI) was 0.0438 ($p < 0.001$), and the relative IDI was 0.8926. The net benefit became significant from a predicted probability $\geq 20\%$; 42 patients were true positive (TP), 101 were false positives (FP) and the NET was 0.080 based on ABCD²-only Model, 36 patients were TP, 63 were FP and the NET was 0.097 based on ABCD²+CRP Model (Figure 2C).

Table 3. Cox regression coefficients for CRP and ABCD² score for cerebro- and cardiovascular events and vascular mortality

Prognostic variables	Parameter estimate	Standard error	p values	Hazard Ratio	95% CI
ABCD2 score ≥ 4	1,35	0,40	0,0008	3,85	1,75 - 8,46
CRP ≥ 3 mg/l	0,98	0,32	0,0025	2,65	1,42 - 4,97

DISCUSSION

The usefulness of a biomarker in clinical medicine depends on whether it gives informations to the clinicians by reliably identifying groups with a specific prognosis. This study produced useful findings for TIA prediction. Using a population-based registry, we prospectively assessed the ability of CRP levels, compared to known risk factors expressed as the ABCD² score, to predict ischaemic events. Routine CRP measurements might be a useful tool for identifying TIA patients who are at a higher risk of ischaemic stroke. Our findings support the concept of a CRP-level cut-off of 3 mg/L may as an helpful predictor of cerebral ischaemic events in TIA patients. However, it is necessary to exclude all patients with concurrent infection or inflammatory disease, which may alter CRP levels and confound the results. Several biomarkers have been evaluated for risk prediction in ischaemic conditions, and CRP was shown to be a powerful predictor of future coronary and cerebral ischaemic events [1-3].

Previous studies demonstrated a strong predictive association between elevated CRP concentrations and future atherothrombotic events (stroke, coronary events and peripheral arterial disease) [24]. High CRP levels represent a strong risk predictor for subsequent ischaemic stroke among patients with preexisting atherothrombotic disease [25]. High CRP levels are associated with intima media thickness, development of atherosclerotic plaques, their progression, rupture and ischaemic stroke [26]. Many possible mechanisms of a direct action of CRP on vascular cells have been reported. CRP plays an important role in the immune response and is implicated in the development and complications of atherosclerosis [2, 6]. High CRP levels may up-regulate adhesion molecules and chemokine expression, which promote vascular inflammation [27]. A meta-analysis of studies with long-term follow-up (> 8 years) showed that the risk for stroke in healthy individuals in the highest quartile of CRP concentrations increased nearly 70% compared with those in the lowest quartile [28].

There has been much interest in identifying prognostic indicators that can be used to determine which patients are at a higher risk of stroke after TIA. The risk of stroke after TIA has been reported to be 2% to 4% at 2 days, approximately 6% at 7 days, and of 10% to 15% at 90 days [13, 31]. Recently, an effort has been made to risk stratify patients who present with TIA using clinical scoring (ABCD system) [14]. This score has the advantage of using easily assessable clinical variables. However, some patients have strokes despite a low predicted risk according to this score. In a systemic review of stroke risk after TIA using the ABCD system, it was estimated that the early risk of stroke varies according to the clinical speciality of the examining physician, the evaluation setting, the method of data extraction and the limited number of outcome events [31]. The ABCD² system is considered to be the best available method for determining which patients are at short-term risk for stroke after TIA although the clinical judgment and the data from multiple sources, such as imaging and biochemistry, must be incorporated into the score. In addition, a recent study showed that the inclusion of brain and carotid imaging into the ABCD² score improved the risk stratification after TIA in secondary care settings [31]. However, the prognostic value of the ABCD-score or the ABCD²-score has been questioned recently [13, 31]. There have been only few studies that evaluated biomarkers exclusively in patients with TIA, however their methodology and evaluated outcomes were different [10, 11]. There was no relationship between CRP and outcome in a single study where the ABCD score and biomarkers for risk-stratification were compared [11]. High-sensitivity CRP serum level predicts further ischaemic events following TIA [10].

The baseline characteristics of the current study (age, gender, and clinical features) were comparable to most of the aforementioned studies [10-14,31]. However our study was not designed to investigate the rationale for decision making in the care of TIA patients. Higher CRP levels in our data are related to a larger number of vascular events and vascular deaths when compared to the ABCD² ≥ 4 score alone. It has to be noted that the higher CRP group was significantly older than the lower CRP group. Moreover, the high rate risk of stroke in our cohort might be due to limited statin use. Statins have pleiotropic effects, including anti-inflammatory actions. Statins reduce plasma CRP levels and this decrease is independent of LDL cholesterol reduction [29]. Although a weak association between cholesterol and stroke has been shown in the past, a decrease in cholesterol concentrations with statins can reduce the incidence of stroke in high-risk populations [9, 33] and in patients with a history of TIA or stroke [30]. There are no large randomised, controlled trials that indicate that lowering of CRP levels should be a secondary prevention strategy in TIA or stroke patients. A recent trial on apparently healthy individuals with high levels of CRP without hyperlipidaemia showed that treatment with statins reduced the incidence of major cardiovascular events [9]. Moreover, the trial provides only limited and indirect information about the role of CRP. A single CRP measurement equal to or greater than 3 mg/L, in patients with TIA, might play an important role in the selection of patients who are at a higher risk of new cerebral ischaemic events.

More importantly, adding CRP levels to the ABCD² score enhanced the prediction of subsequent cerebral ischaemic events. Patients who are at the highest risk are better identified by combining CRP levels with ABCD² scores. This in turn may lead to the development of new treatment strategies for primary stroke prevention in patients identified as being at risk for developing cerebro-vascular disease. In conclusion, the additional use of CRP levels for the risk assessment in TIA patients improves risk definition in terms of the ABCD² score

alone. Routine CRP measurement should be used in TIA patients who do not have concurrent infection or inflammatory comorbidities and might benefit from more aggressive secondary prevention strategies or from a lowering of CRP levels, which may be achieved either with or without medication [34-37].

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Chapter 57

THE GROWING PREVALENCE OF ATRIAL FIBRILLATION, ESPECIALLY ON STROKE UNITS

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ABSTRACT

Atrial fibrillation (AF) is the most common sustained cardiac rhythm disorder and is associated with a 4- to 5-fold increase in the risk of stroke and systemic embolism. The dominant position of AF in present stroke units may be understood as in line with its high prevalence but also with the functional impairment of strokes caused and increased neurological severity at presentation.

Across the years different series have expressed heterogeneous results on the relative frequency of cardioembolism as etiology of ischemic stroke. Nonetheless, a clear uprising tendency may be observed with an almost two-fold increase in the last 25 years. Many factors concur to this development but mainly the aging of populations. Notably, the relationship of AF with age extends beyond its higher frequency to the greater stroke risk involved. This factor together with the improvement in control of classical vascular risk factors has produced an exponential breakthrough in the presence of AF in current stroke units. Furthermore, despite the efficacy of vitamin K antagonists, fear of hemorrhagic complications along with doubts on “low-risk” patients and difficulty in accessing regular analytical control greatly impaired its adequate usage.

The major factors promoting the ascent of AF are mostly set to worsen in the upcoming years. The new generation of anticoagulants is a new determinant in this equation but their ultimate impact is yet to be ascertained.

INTRODUCTION

Cerebrovascular diseases comprise one of the main causes of mortality and morbidity worldwide. Nonetheless, the precise definition of the etiopathogenic underlying mechanism is

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crucial to guide acute medical care and, more significantly, the secondary prevention strategy (1-3). Cardioembolism has always been an important source of ischemic stroke, however it has shown quite heterogeneous frequencies throughout different series ranging from 15% to over 40% (1-6). Although many cardiac conditions can account for systemic embolism one pathology arises as the undisputed leader causing ischemic stroke: Atrial Fibrillation (AF). Atrial fibrillation (AF) is the most common sustained cardiac rhythm disorder and is associated with a 4- to 5-fold increase in the risk of stroke and systemic embolism (3). Ultimately, this arrhythmia has been recognized as one of the major medical challenges in modern society and its clinical, social and economic aspects are all set to worsen over the coming decades, especially considering its increasing frequency with age (3, 7).

The dominant position of AF in present stroke units may be understood in line with its high prevalence but also with the clinical features particular to the ischemic strokes caused. In fact, although cardiac emboli can take any size they frequently have larger dimensions than in other causes of stroke (1) leading to more severe strokes with lower frequencies of hospital discharges to asymptomatic patients, greater mortality, as well as higher short-, medium- and long-term recurrence rates (5, 8-10). Moreover, cardioembolic strokes typically present with a sudden onset to maximal deficit (< 5 minutes) which is present in 47-74% of cases and decreased level of consciousness at onset in 19-31% of cases (9, 11-14) both features directly causing higher admission rates to stroke units.

Altogether, cardioembolism and particularly AF, has gradually been assuming a more and more prevalent position in the patients admitted to contemporary stroke units worldwide.

The aim of this chapter is to briefly review the impact of AF in current stroke units as well as analyzing possible trends in its prevalence.

PREVALENCE OF CARDIOEMBOLISM IN STROKE UNITS

Across the years different series have expressed heterogeneous results on the relative frequency of cardioembolism as etiology of ischemic stroke. In western countries a clear tendency can be observed to the uprising presence of AF (figure 1). In the late 80's/early 90's cardioembolism was held responsible for less than 20% of ischemic strokes (15-18). These figures increased to under 30% in the first decade of the 21st century (19, 20) and values of over 30% in the second decade (4, 6).

The comparative studies of Carrera et al. and Arboix et al. both document an almost two-fold increase in the frequency of cardioembolism in the past 25 years (from 17.5% in 1979-1987 to 27.8% in 1996-2003 and 14.6% in 1986-1992 to 27.6% in 1999-2004, respectively)(19, 20). In parallel, AF has also shown a significant increase (from 20.8% to 29.3% in the latter study)(19).

A few conditions may account for this evolution. Firstly and more importantly the indirect effect of population aging. The increment of AF with age is well established with reported frequencies of almost 15% in the population over 80 years old (3, 21-23). Moreover the relationship of age with AF extends to the increased risk of stroke. This data has been translated into clinical recommendations and has motivated a review of previous cardioembolic risk scores. In fact, the relative weight of age (particularly when over 75 years old) has risen in the recently created CHA₂DS₂VASc score and is now considered to be

equivalent to the risk escalation caused by a previous ischemic cerebrovascular event (7). Another factor relates to the worldwide campaigns to monitor and control classic vascular risk factors, such as high blood pressure, dyslipidemia, smoking or elevated blood glucose. The impact of these measures now begin to be visible with a reduction of ischemic events related to those risk factors. This ultimately promotes aging of populations which in turn perpetuates the exponential increase in the relative presence of AF as etiology of stroke. Furthermore, up to a few years ago only one valid strategy stood for stroke prevention: vitamin K antagonists. This class of drugs, albeit its unequivocal efficacy, was massively underused (6, 24-26) mostly due to doubts on “low-risk” patients, fear of hemorrhagic complications and difficulty in accessing regular analytical control(8, 27, 28).

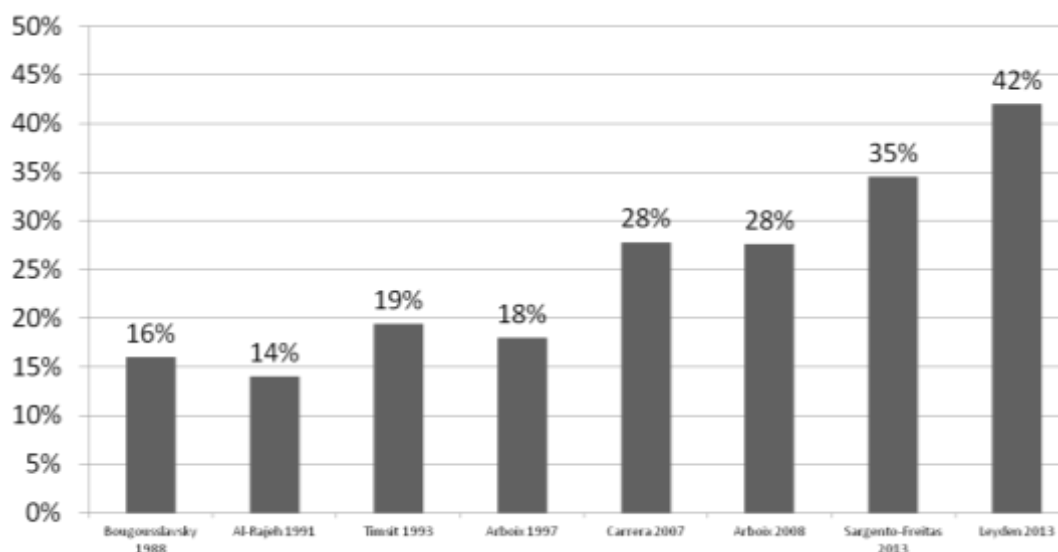


Figure 1. Histogram representing the relative frequency of cardioembolism across different studies. The results are shown chronologically according to the year of publication.

A SINGLE CENTRE EXPERIENCE

To evaluate the frequency of cardioembolism in our centre (a tertiary hospital in Portugal) we performed a crosssectional analysis to all consecutive patients admitted with the diagnosis of ischemic stroke during the year of 2010 (6). Notably, all patients with ischemic stroke are admitted in our centre. We chose to exclude transient ischemic attacks as they are not systematically admitted. The etiology of stroke was defined according to the TOAST criteria (29). In patients with the diagnosis of AF (permanent, persistent or paroxysmal) previous to the stroke we analyzed antithrombotic options.

We had a total of 631 patients admitted due to ischemic stroke. Mean age 73.2 years (SD: 12.4) and 342 (55.2%) male. Cardioembolic stroke was the most frequent with 218 patients (34.5%), small vessel occlusions was documented in 18.2%, large artery atherosclerosis in 25.8%, other causes in 7.0% and the cause remained undetermined in 14.4%. From the patients with cardioembolic stroke 199 (91.3%) were due to, 5 (2.3%) to prosthetic valve, 3

(1.4%) to cardiac insufficiency, 4 (1.8%) a embolic valvulopathy, 2 (0.9%) to acute myocardial infarction and 1 (0.5%) to patent foramen ovale. The frequency of AF in the whole stroke population increased to 32.2% (203 patients) adding 4 patients with AF classified as undetermined due to multiple etiologies.

Among the ischemic strokes caused by AF, the vast majority, (65.3%, 130 patients) had its diagnosis previous to the stroke. From those, only 26.9% (35) were on vitamin K antagonists and only 8.5% (11) showed INR within the therapeutic range (figure 2).

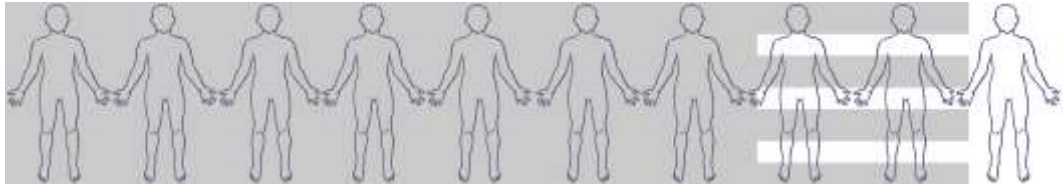


Figure 2. schematic representation of the antithrombotic options in the patients with the diagnosis of AF prior to the ischemic stroke. In grey are represented patients on antiplatelets (63.1%); in stripes patients on vitamin K antagonists outside therapeutic INR range (18.4%); in white those on therapeutic INR range (8.5%).

These results confirm the higher prevalence of cardioembolic stroke reported in recent literature. However, the antithrombotic options observed prior to stroke rise significant concern. In fact, although similar to other series (24, 25) they are contrary to the recent guidelines of the European Society of Cardiology (3) that recommend disconsidering the use of antiplatelet agents as cardioembolic prophylaxis in primary and secondary prevention. The low number of patients with AF and ischemic stroke presenting with therapeutic INR confirms the efficacy of vitamin K antagonist while on therapeutic dosage and is once again a possible sign of under usage of these therapeutic agents.

FUTURE PERSPECTIVES

The clinical, social and economic aspects are all set to worsen over the coming decades³. Looking at the main factors that sustained the ascent of AF in stroke units it is unlikely to presume a change in the upcoming years. One factor seems to promote this above all: age. Populations are expected to grow older in the near future and this will render a continuous increase in relative frequency of AF. On the other hand, one aspect is subject to change: therapeutic approach. Indeed a new generation of anticoagulants has been approved for stroke prevention (30-32). Their improved safety profile has shifted the paradigm of “low-risk” and the absent requirement of periodic analytical control has enhanced access to efficient therapy. Nonetheless concerns remain on their short-acting mechanisms of action and therapeutical approach to patients with stroke whilst taking these medications. We enthusiastically await to see their effect in the relative frequency of AF in the stroke units of the years to come.

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Chapter 58

N-TERMINAL PROBRAIN NATRIURETIC PEPTIDE AS A BIOMARKER OF CARDIOEMBOLIC STROKE

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ABSTRACT

Even after an extensive etiological investigation in about one third of ischemic strokes it is not possible to establish a cause. A fraction of these strokes could have been due to an episode of paroxysmal atrial fibrillation that was not diagnosed. Studies show that the current available methods to diagnose atrial fibrillation have a low sensitivity. Therefore, it is important to consider new ways to diagnose paroxysmal atrial fibrillation. New methods to diagnose atrial fibrillation include the use of biomarkers. One of the substances that has been pointed as a possible biomarker in this context is the N-terminal of the brain natriuretic peptide (NT-proBNP).

NT-proBNP serum levels in patients with cardioembolic stroke are higher than in patients with non-cardioembolic stroke. Cut-off points of serum NT-proBNP levels with a high sensitivity and specificity for the diagnosis of paroxysmal atrial fibrillation in the first 72 hours after ischemic stroke have been established and analysed in patients in patients with cryptogenic stroke. Measuring NT-proBNP levels in patients with cryptogenic stroke could help to select which patients benefit the most from long term heart rhythm monitorings looking for paroxysmal atrial fibrillation.

Keywords: NT-proBNP, biomarker, paroxysmal atrial fibrillation, diagnosis, stroke

INTRODUCTION

Paroxysmal atrial fibrillation (pAF) is reported to have the same risk to cause ischemic stroke as permanent or persistent atrial fibrillation [1].

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However its often short duration and lack of accompanying symptoms can difficult its diagnosis making it clinically under detected [2]. Due to the importance of diagnosing paroxysmal atrial fibrillation in the context of ischemic stroke all patients should do at least one 12 lead ECG during the etiological workup of stroke, as recommended by both the American Heart Association and the European Stroke Organization [3, 4]. In patients with an undetermined stroke etiology and when a cardioembolic stroke etiology is suspected, it is also recommended to perform a 24 hours ECG recording (Holter) [3, 4]. The 24 hours ECG recording has a higher sensitivity for the diagnosis of paroxysmal atrial fibrillation than a routine 12 lead ECG. In a systematic review [5] the detection rate of paroxysmal atrial fibrillation by a 24-72 hours ECG recording was 4.6%. Serial ECG assessments within the first 72 hours of an acute stroke significantly improve detection of AF [6]. Longer recordings such as with a 7-day ambulatory ECG monitoring using an event-loop recording (ELR) device detected paroxysmal atrial fibrillation in more 6 to 8% of patients after a non-diagnostic 24 hours ECG monitoring [7]. The use of 7 days event-loop recorders at 0, 3 and 6 months after stroke onset detected paroxysmal atrial fibrillation in 14% of patients with an initial negative 24 hours ECG monitoring [5]. Nevertheless in order to register events using this device it is necessary to have the patient's collaboration and periods of asymptomatic atrial fibrillation are therefore not noticed. A Mobile Cardiac Outpatient Telemetry during a period of 21 days in patients with cryptogenic transient ischemic attack or stroke resulted in a paroxysmal atrial fibrillation detection rate of 23% [8].

Currently, about one third of ischemic strokes after a comprehensive investigation are classified as having an undetermined cause. It is possible that a fraction of these strokes named as cryptogenic may have been due to an undetected episode of atrial fibrillation [9]. Due to low sensitivity of the current available methods to detect paroxysmal atrial fibrillation, alternative ways to detect this arrhythmia in cryptogenic stroke have been considered, namely the use of biomarkers. One of the substances that are being studied as possible biomarkers of cardioembolic stroke related to atrial fibrillation is the N-terminal of the brain natriuretic peptide (NT-proBNP).

BRAIN NATRIURETIC PEPTIDE AND N-TERMINAL PROBRAIN NATRIURETIC PEPTIDE

Biomarker is defined as a molecular, biological, or physical characteristic that indicates a specific physiologic state. It is used in clinical practice to identify risk for disease, diagnose disease and its severity, guide intervention strategies, and monitor patients response to therapy [10]. Biomarkers have been progressively recognized as important diagnostic tools. Ideally, a biomarker should be highly sensitive, specific, accessible, accurate, reproducible by an analytical method, cost-effective and have a result that can be easily interpreted by a physician [11].

NT-proBNP is part of a group of natriuretic peptides, phylogenetically conserved along time. Included in this group of peptides are also atrial natriuretic peptide (ANP), natriuretic peptide type C, urodilatin and the *Dendroaspis* natriuretic peptide [12].

The NT-proBNP is initially produced as a prepropeptide of 143 amino acids. This prepropeptide is proteolytic cleaved into a non-active N-terminal fragment composed by 10

amino acids designated proBNP. ProBNP after secreted is divided in two fractions by proteolytic enzymes. These two fractions are BNP that is biological active (aa 77-108) and the N-terminal-proBNP (NT-proBNP) (aa 1-76) without biological activity [13]. BNP and NT-proBNP concentrations can be determined in blood samples [14]. The half-life of NT-proBNP is superior to the half-life of BNP (120 minutes versus 22 minutes) [15]. It is due to this difference in half-life that most essays measure NT-proBNP instead of BNP. Although it was initially isolated in porcine brain in 1988 [16], explaining its designation as brain natriuretic peptide, subsequent experiences showed that it also had a cardiac production [17]. Currently it is considered that the heart is its main production site. In the brain, NT-proBNP is mainly produced in the hypothalamus. The cerebral cortex, thalamus, pons have also been pointed as possible production sites [18]. NT-proBNP in normal situations does not cross the blood-brain barrier [19].

In the heart, NT-proBNP can be produced by both atria and ventricles [20]. After being produced NT-proBNP is mainly secret in “bursts”. Its storage in granules is minimal [21]. NT-proBNP seems to be more present in the atria rather than in the ventricles. However, due to the larger ventricular mass, 70% of all NT-proBNP is produced by the ventricle in normal conditions [22]. The main stimulus for the synthesis and excretion of NT-proBNP in the heart is myocytes stretching, mainly in the context of volume [23] or cardiac pressure overload [24]. After stretching there is a rapid activation, within hours, of NT-proBNP gene expression [25]. Certain hormones (catecholamines, angiotensin II and endothelin-1) may stimulate the production of NT-proBNP through paracrine or endocrine mechanisms. [26]

BNP acts by an endocrine mechanism. It has an important role in the cardiovascular homeostatic regulation and in volume control. Its biological effects include natriuresis, diuresis, vasodilatation, inhibition of the renin-angiotensin-aldosterone axis and of the sympathetic nervous system [27]. In the heart, BNP has a lusitropic and antifibrotic effect [28]. NT-proBNP is cleared from circulation through the kidney by direct filtration and passive excretion [29].

Serum NT-proBNP and BNP levels in healthy individuals increase with age and are higher in women than in men [30]. Ninety percent of young adults have NT-proBNP levels below 70 pg/mL [31]. BNP plasmatic levels correlate with left ventricular mass. NT-proBNP is currently used as a marker of left ventricular dysfunction and of prognosis in patients with congestive heart failure and in acute ischemic heart disease [32]. High NT-proBNP serum levels have been found in patients with renal failure, anemia, acute pulmonary embolism, pulmonary hypertension, sepsis, mitral and aortic regurgitation and dysrhythmias such as atrial fibrillation [33, 34].

Without structural heart disease, patient with lone paroxysmal atrial fibrillation have increased NT-proBNP [35]. Increased plasmatic levels of NT-proBNP have been found in patients with permanent or paroxysmal atrial fibrillation with preserved left ventricular systolic function [36]. A return to baseline of NT-proBNP values after electric cardioversion of atrial fibrillation to sinus rhythm was documented [37]. NT-proBNP has been shown to be a remarkable predictor of incident AF in the general population, independently of any other previously described risk factor [38]. Recent studies that suggest that NT-proBNP is a predictor of atrial fibrillation following cardiac surgery [39-41] and successful cardioversion [42].

There is currently no established explanation for the increase of NT-proBNP in the context of atrial fibrillation. In the isolated atria tissue of rat, alpha1-adrenergic stimulation

with phenylephrine induces genetic expression of BNP [43]. It is unknown if alpha1-adrenergic stimulation results in an increase in the synthesis of NT-proBNP in patients with atrial fibrillation. A study that analysed human right atrial tissue found that persistent atrial fibrillation was associated to a high expression of proBNP mRNA. However, patients with paroxysmal atrial fibrillation did not have changes in proBNP gene expression [44].

NT-PROBNP IN ACUTE ISCHEMIC STROKE

Although NT-proBNP presence was first described in the brain tissue, information regarding NT-proBNP in ischemic stroke is small. Some studies showed an acute increase in NT-proBNP and BNP serum levels in acute ischemic stroke [19, 45-53]. These studies initially aimed to determine if NT-proBNP or BNP could be used as prognostic markers of acute ischemic stroke along with other markers of myocardial injury such like troponins. It was suggested that it could be used as a marker of long term bad prognosis. It was then noticed that patients with acute ischemic stroke tended to have high levels of NT-proBNP and BNP. Four possible explanations for the increase of NT-proBNP and BNP during acute ischemic stroke were initially proposed in these studies:

- 1 Patients with acute ischemic stroke frequently have several vascular risk factors and may have an associated acute or chronic heart failure. Therefore, the increase in NT-proBNP levels could be due to ventricular dysfunction;
- 2 The increase in NT-proBNP levels could be due to atrial fibrillation. Atrial fibrillation is a major risk factor for ischemic stroke and a known cause of NT-proBNP increase;
- 3 As the brain is a site of BNP and NT-proBNP production, although it only produces a small amount of these peptides it is possible that after an acute ischemic lesion there is a release of BNP and NT-proBNP that is measurable in the serum.
- 4 BNP has an inhibitory action in the central and peripheral sympathetic nervous system. BNP could be increased in acute ischemic stroke as a response to the increase sympathetic activity that occurs after stroke

Serum NT-proBNP levels were further analysed in patients with acute ischemic stroke [54]. Patients with cardioembolic ischemic stroke were found to have mean concentrations of NT-proBNP which were significantly higher than in patients with non-cardioembolic stroke. An association between serum NT-proBNP levels and the extent or location of ischemic stroke or blood pressure measurements was not found. NT-proBNP was found to have a good accuracy for the diagnosis of cardioembolic stroke in general (Area under the curve (AUC) of 0.77 ± 0.12) and a very good accuracy for the diagnosis of cardioembolic stroke related to atrial fibrillation (AUC of 0.92 ± 0.06). For the diagnosis of cardioembolic stroke related to atrial fibrillation, two cut-off points of NT-proBNP with a high sensitivity and specificity were determined from the analysis of receiver operating characteristic curves - 265.50 pg/mL (sensitivity 94.4%, specificity 72.9%, positive predictive value 56.6% and negative predictive value 97.2%) and 912.0 pg/mL (sensitivity 55.5%, specificity 97.9%, positive predictive value 90.9% and negative predictive value 83.9%).

Most of the studies that measured NT-proBNP as a biomarker of cardioembolic stroke measured this peptide during the first 12 to 24 hours after the onset of symptoms of ischemic stroke. However, there was no clear evidence that this was the best time to measure it. In a study that aimed to determine NT-proBNP levels within 24, 48 and 72 hours after ischemic stroke and to analyse its variation [55], in all included stroke patients and in those with cardioembolic and noncardioembolic stroke (including undetermined causes), values of NT-proBNP were highest in the first 24 to 48 hours after ischemic stroke with no statistically significant difference between those two time points and had a statistically significant reduction 72 hours after stroke onset.

However, the value of the area under the curve for the diagnosis of cardioembolic stroke related to atrial fibrillation was similar in the three time points. This suggested that the measurement of NT-proBNP levels with the first 72 hours after ischemic stroke had a similar diagnostic accuracy for the diagnosis of cardioembolic stroke. The form of release of NT-proBNP from the heart may explain this time course. Normally atrial and ventricle myocytes express BNP genes that code for ProBNP. ProBNP is stored in atrial and ventricular granules. In the first two days following stroke, the precursor molecules, pre-produced proBNP that are stored in atrial myocytes are secreted as BNP and NT-proBNP after the haemodynamic effects of atrial fibrillation or atrial flutter [56]. This causes the high levels of NT-proBNP that are observed. At Day 3, NT-proBNP levels may start to decrease due to a depletion of stored proBNP.

NT-proBNP cut-off levels for the diagnosis of atrial fibrillation were further validated in patients with cardioembolic stroke and in patients' with an initial cryptogenic stroke that were followed prospectively. Among 264 ischemic stroke patients NT-proBNP serum levels were measured within 72 hours of stroke onset [57]. In cryptogenic stroke patients, 24 hour Holter monitoring was used to look for pAF within the first week, three and six months after admission. First, patients with a defined etiology were used to construct a Receiver Operating Characteristic (ROC) curve for the diagnosis of AF. From this curve, the sensitivity and specificity of pre-established cut-off points for the diagnosis of atrial fibrillation were calculated. The cut-off points were then evaluated in patients with cryptogenic stroke. The Area Under the Curve of the Receiver Operating Curve of NT-proBNP for the diagnosis of AF was 0.91, 95% CI (0.87-0.95).

Using multivariate analysis NT-proBNP was independently associated to AF (OR – 2.65, 95% CI 1.57-4.45; $p < 0.0001$), after adjusting for age, sex, left ventricular hypertrophy, atrial dilatation and systolic dysfunction. 80 patients had a cryptogenic stroke. In 17, pAF was found during follow-up.

In these patients, the AUC for the diagnosis of paroxysmal atrial fibrillation was 0.83. The cut-off point of 265.5 had a sensitivity of 88.2% and a specificity of 61.9%. The cut-off point of 912 pg/mL had a sensitivity of 47.1% and a specificity of 88.9%. NT-proBNP had a good accuracy to predict pAF in patients with cryptogenic stroke. NT-proBNP levels at the time of stroke onset were helpful to identify paroxysmal atrial fibrillation in the follow-up of patients with an undetermined stroke etiology.

Further studies are needed to support and validate the defined cut-offpoints. A clinical trial of anticoagulation in cryptogenic stroke patients with increased NT-proBNP could be helpful.

CONCLUSION

In patients with cryptogenic stroke, detecting paroxysmal atrial fibrillation is particularly important as it changes the therapeutic decision, leading to the prescription of anticoagulants instead of antiplatelet agents. Cryptogenic stroke patients are several times submitted to costly electrophysiological investigations. NT-proBNP measurements could be helpful to guide or eliminate further electrophysiologic monitoring in patients with cryptogenic ischemic stroke. In patients with cryptogenic stroke with uneventful echocardiograms an increase in NT-proBNP may point to the presence of paroxysmal AF and lead to further cardiac electrophysiological investigations.

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Chapter 59

FROM CRYPTOGENIC STROKE TO PAROXYSMAL ATRIAL FIBRILLATION

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ABSTRACT

Ischemic stroke, the most prevalent type of stroke is, frequently, the result of large artery atherosclerosis, small vessel disease or cardioembolism.

However, the etiology remains unclear in up to 25 to 30% of stroke patients, despite a complete initial work-up. In some of them, paroxysmal atrial fibrillation (PAF), a condition that carries an equally high stroke-risk as persistent or permanent atrial fibrillation, is a likely contributing cause.

There are some clinical characteristics but also imaging, electro and ecocardiographic findings that are suggestive of a cardiac source of embolism. In this chapter we will discuss in detail the features that support the suspicion of a cardioembolic stroke specifically due to PAF.

Different kind of noninvasive and invasive detection methods have been developed in the last few years, to monitor cardiac rhythms for long time periods. They are, and probably will be in the near future, an important tool to identify PAF in cryptogenic stroke patients. It remains to be established, however, the criteria that should be used to select the patients that are more likely to benefit from a closer cardiac monitoring.

INTRODUCTION

Ischemic stroke, the most prevalent type of stroke, is caused by thrombotic or embolic occlusion of cerebral arteries. Embolism, in turn, can result from an arterial or cardiac source. The most relevant etiopathogenic subgroups, as reflected by the TOAST classification [1], are large artery atherosclerosis, cardioembolism and small vessel disease.

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In many patients, clinical history, physical examination and routine diagnostic tests (electrocardiogram, cervical ultrasonography and transcranial doppler, neuroimaging studies, blood analysis) are enough to easily determine the presumed etiology [2].

Some stroke patients are not adequately investigated. In those with an incomplete evaluation during hospitalization, the overall cumulative death rate (25,5% within one year) is highest when compared with those with undetermined etiology despite extensive work-up. [3].

The identification of the etiology and mechanism involved in ischemic stroke is a great challenge for the clinician but is essential for planning adequate secondary prevention. Nevertheless, a relevant number of patients (25 to 30%) (4) is considered to have a stroke of unknown cause, despite full investigation – the so-called cryptogenic stroke .

Although there is a considerable disagreement among experts regarding the extent of cardiac testing in stroke patients [5], searching for a cardiac etiology is a relevant, sometimes determinant, part of the investigation. In the presence of a cryptogenic stroke, one of the major suspects is paroxysmal atrial fibrillation (PAF) and a presumed cardioembolism should not be excluded just because we can not find any structural cardiac abnormality.

In fact, several small studies of long-term cardiac monitoring have suggested that 6,4% to 21,3% of patients with ischemic stroke of unknown cause may have PAF as a likely contributing cause [6].

PAROXYSMAL ATRIAL FIBRILLATION (PAF)

AF is an atrial tachyarrhythmia characterized by predominantly uncoordinated atrial activation with consequent deterioration of atrial mechanical function [7].

It is the most common sustained rhythm disorder and is, probably, a self-promoting disease in which progressive electrophysiologic remodeling and anatomical changes in the atria play a role in the perpetuation of AF [8]. The pathogenesis is thought to involve an interaction between initiating triggers, often in the form of firing ectopic foci located inside one or more pulmonary veins and an abnormal atrial tissue substrate capable of maintaining the arrhythmia [9]. It is, therefore, understandable that PAF frequently evolve to persistent or permanent AF . PAF prevalence is estimated to comprise between 25 and 62% of cases of AF seen by practitioners in both hospital and primary care setting [10] . The first symptomatic episode may follow many silent ones [11] and thus, reported prevalence is, probably, underestimated as most epidemiological studies depend on symptomatic episodes [10] and asymptomatic AF is common.

On the other hand, PAF carries an equally high stroke-risk as persistent or permanent AF [12] and the frequency and duration of PAF are thought to be associated with that risk [13]. In the TRENDS study [14], a prospective, observational study enrolling patients with ≥ 1 stroke risk factor (heart failure, hypertension, age ≥ 65 years, diabetes, or prior thromboembolism) receiving pacemakers or defibrillators, it was noticed that an atrial tachycardia/AF burden ≥ 5.5 hours on any of 30 prior days appeared to double thromboembolic risk.

In the last few years, different kind of noninvasive and invasive detection methods have been developed: event recorders, external loop recorders/mobile cardiac outpatient telemetry (MCOT), implantable loop recorders, implantable therapeutic devices.

With the improvement in technology it is now possible to perform cardiac monitoring for long periods of time. Furthermore, these devices are so sensitive that it allows the detection of rare, very brief, asymptomatic episodes of AF (often referred to as occult AF) that likely would have eluded detection using standard in-patient telemetry or Holter monitoring [15].

PAF was detected in 27 of 156 (17.3%) cryptogenic stroke patients using a 21-day mobile cardiac outpatient telemetry [16].

In the SMART registry [17] approximately 1 in every 9 patients with cryptogenic ischemic stroke was found to have new PAF within 30 days.

The CRYSTAL AF trial [4] was a randomized prospective single-center cohort study to evaluate a implantable subcutaneous cardiac monitor to long-term monitoring for AF detection in patients with cryptogenic stroke. The preliminary data was published in July/13. The cardiac event recorder was eligible for implantation in approximately one third of patients and PAF was detected in 27,3% of these cryptogenic stroke patients. These results should be cautiously interpreted, given the notorious small number of eligible patients.

Until this precise moment some questions remain partially unanswered when facing a cryptogenic stroke:

- Which parameter/combination of parameters rise suspicion of PAF involvement in stroke etiology?
- Which group of patients should be submitted to close cardiac monitoring after a cerebral ischemic event of unknown cause?

In this article we will review the main features associated with stroke due to paroxysmal atrial fibrillation.

FEATURES THAT SUPPORT THE SUSPICION OF A CARDIOEMBOLIC ETIOLOGY DUE TO PAF IN CRYPTOGENIC STROKE

A – Absence of a More Plausible Etiology for the Stroke

A1 – Exclusion of a «vascular etiology»

After a complete work-up, a paroxysmal AF etiology for the ischemic stroke should remain the first suspect in the absence of a more plausible etiology for the event, specially a «vascular etiology». We use the same definition reported by Laurent Suissa et al. [18] and so, for «vascular etiology» we mean cervical and/or cerebral symptomatic atherosclerosis (intra and/or extracranial stenosis $\geq 50\%$), clinico-radiological lacunar syndrome or other vascular cause for the stroke (dissection, etc).

Whether a carotid stenosis of less than 50% is a marker of atherosclerosis or also a cause of stroke [19], specially in the presence of heterogeneous and irregular plaques, is a matter of debate.

A2 – Exclusion of Other Causes

Stroke may result from inflammatory diseases, migraine and other vasoconstrictive conditions, connective tissue disorders, coagulopathy, infection or malignancy. These entities should be considered and excluded.

B - Clinical Features

Strokes due to cardiac embolism are generally associated with poor prognosis, short and long-term recurrence, higher in-hospital mortality and a higher index of fatal recurrence versus other causes of stroke [5].

Atrial fibrillation, in particular, an age-related condition, is associated with a greater volume of more severe baseline hypoperfusion, leading to higher infarct growth and worse stroke outcomes [20]. When an ischemic stroke is under investigation we should keep in mind that a cardiac source typically produces any kind of non-lacunar syndrome presentation. That is, a total or partial anterior circulation syndrome (TACS or PACS) or a posterior circulation one (POCS). Clinical noncortical lacunar strokes and proven lacunar infarcts are etiologically and pathophysiologically distinct from other infarcts and are caused, usually, by cerebral small vessel disease [21]. Gan et al. reported that 75% of patients with radiological confirmed lacunar infarction had a lacunar mechanism of infarction. Cardioembolism was presumed in only 5% of these patients [22]. Although a sudden onset to maximal deficit (less than 5 minutes) may suggest a cardiac source of emboli, it is not specific, as this pattern overlaps with other stroke causes [23]. A rapid regression of the symptoms occur in 4,7 to 12% of the cardioembolic stroke patients which may be due to distal migration of the embolus and recanalization of the vessel [2, 24].

Isolated aphasia was suggested to be an independent sign of AF in patients with a transient ischemic attack (TIA) or PACS [25]. Visual-field abnormalities and neglect are also clinical features that support the suspicion of an embolic source [26].

In the vertebro-basilar circulation we may think of embolism in the presence of Wallenberg syndrome (usually due to an occlusion of the postero-inferior cerebellar artery), top of basilar artery syndrome or a clinical deficit in the territory of the posterior cerebral artery. Multilevel infarcts and cerebellar infarcts are also more common in cardiac source of emboli [2]. Seizures at onset are not specific of cardioembolism but are more likely to occur from embolism to distal cortical brain tissue compared to small vessel disease infarcts on deep locations [23].

Another feature that may be related with cardiac embolism is the detection of previous chronic treatment with antiarrhythmic drugs. We found that association (Table 1) in our cryptogenic stroke patients in which a PAF was identified during a 6-months follow-up period (unpublished data). We speculate that this finding may be related with clinical previous detection of that arrhythmia but, for some reason, the decision was not to treat with anticoagulants.

C – Cardiac Findings

C1- Electrocardiography

Ischemic stroke patients usually perform a baseline ECG in the emergency setting and later one, sometimes more, during their stay in the hospital. Those who are admitted in the stroke unit are under continuous cardiac monitoring for some days and nurses should be trained in the detection of any kind of atrial arrhythmia. We also recommend to check for all available ECG/Holter monitoring carried out before the stroke.

Table 1. Baseline characteristics of cryptogenic stroke patients registered between June 2010 and December 2012 in our stroke unit. During a 6-months follow-up period PAF was identified in 31,9%

	All patients (n=113)	AF unidentified (n=87)	AF identified (n=36)	<i>p value</i>
Male sex	53 (46,9%)	40 (51,9%)	13 (36,1%)	0.116
Patients > 75 years	54 (47,8%)	29 (37,7%)	25 (69,4%)	0.002
Hypertension	91 (80,5%)	59 (76,6%)	32 (88,9%)	0.125
Diabetes mellitus	30 (26,5%)	19 (24,7%)	11 (30,6%)	0.510
Dyslipidemia	59 (52,2%)	42 (54,5%)	17 (47,2%)	0.468
Overweight	11 (15,3%)	7 (15,6%)	4 (14,8%)	0.1
Alcoholism	10 (8,8%)	7 (9,1%)	3 (8,3%)	1.0
Current or past smoker	36 (31,9%)	9 (11,7%)	1 (2,8%)	0.165
Congestive heart failure	5 (4,4%)	2 (2,6%)	3 (8,3%)	0.325
Coronaryopathy	13 (11,5%)	8 (10,4%)	5 (13,9%)	0.752
Arterial peripheral vascular disease	4 (3,5%)	2 (2,6%)	2 (5,6%)	0.591
Arterial systemic disease	33 (29,2%)	23 (29,9%)	10 (27,8%)	0.820
Previous medication: digoxin	2 (1,8%)	0	2 (5,6%)	0.1
Previous medication: sotalol, amiodarone or CCB	10 (8,8%)	2 (2,6%)	8 (22,2%)	0.002
Previous medication: beta-blockers	13 (11,5%)	6 (7,8%)	7 (19,4%)	0.110
Cerebrovascular US: slight or no atherosclerosis	94 (83,2%)	61 (79,2%)	33 (91,7%)	0.99
Left atrial “smoke” in TEE	5 (9,4%)	1 (2,7%)	4 (25%)	0.025
Left atrial enlargement	27 (42,9%)	9 (22,5%)	18 (78,3%)	<0.001
ECG: SVES	13 (11,5%)	9 (11,7%)	4 (11,1%)	1
Holter ECG: frequent/ very frequent SVES	46 (40,7%)	35 (45,5%)	11 (30,6%)	0.133
Cortical stroke	95 (85,6%)	61 (79,2%)	34 (100%)	0.002
Hemorrhagic transformation	13 (11,5%)	6 (7,8%)	7 (19,4%)	0.110
CT spontaneous hyperintensity	23 (20,4%)	17 (22,1%)	6 (16,7%)	0.506
Previous cortical stroke	34 (30,1%)	14 (41,2%)	20 (58,8%)	<0.001
Previous and current cortical stroke	96 (85,7%)	35 (100%)	61 (79,2%)	0.004
New stroke during follow up	7 (6,2%)	3 (3,9%)	4 (11,1%)	0.207

X² tests were used to compare variables; variables with statistical significance ($p < 0.05$) in bold.
Preliminary data.

CCB: calcium channel blockers; US: ultrasonography; TEE: transesophageal echocardiogram; SVES: supraventricular extrasystoles.

Serial ECG performed in the first 72 hours seems to increase detection almost 3-fold, with half of the new-onset AF being detected on initial ECG and the rest on serial ECG assessments [27].

The 24-hour ECG recording (Holter monitoring) can also play a role in PAF detection. Tagawa et al. reported that PAF was newly identified in 8,4% of stroke patients using this diagnostic testing [28].

The presence of premature atrial complex on ECG was identified and suggested as an independent predictor of PAF detection [16]. In patients with acute ischemic stroke, frequent atrial premature beats ($\geq 70/24$ hours) are a marker for individuals who are at greater risk to develop or have paroxysmal AF [29].

Paroxysmal supraventricular tachycardia was recently proposed [30] as an independent risk factor for ischemic stroke. Whether this risk is related with a higher susceptibility to develop paroxysmal atrial fibrillation is unknown.

C2- Echocardiography

In our own study, left atrial dilation (Figure 1) was strongly associated with paroxysmal atrial fibrillation. The same occurred with spontaneous echo contrast (SEC) in left atrium (LA) and/or left atrial appendage (LAA), also known as «smoke», a relatively frequent finding on transesophageal echocardiography (TEE) but rarely detected on transthoracic echocardiography (TTE) [31], and known to be associated with conditions favoring stasis of left atrial blood (32) (Figure 2).

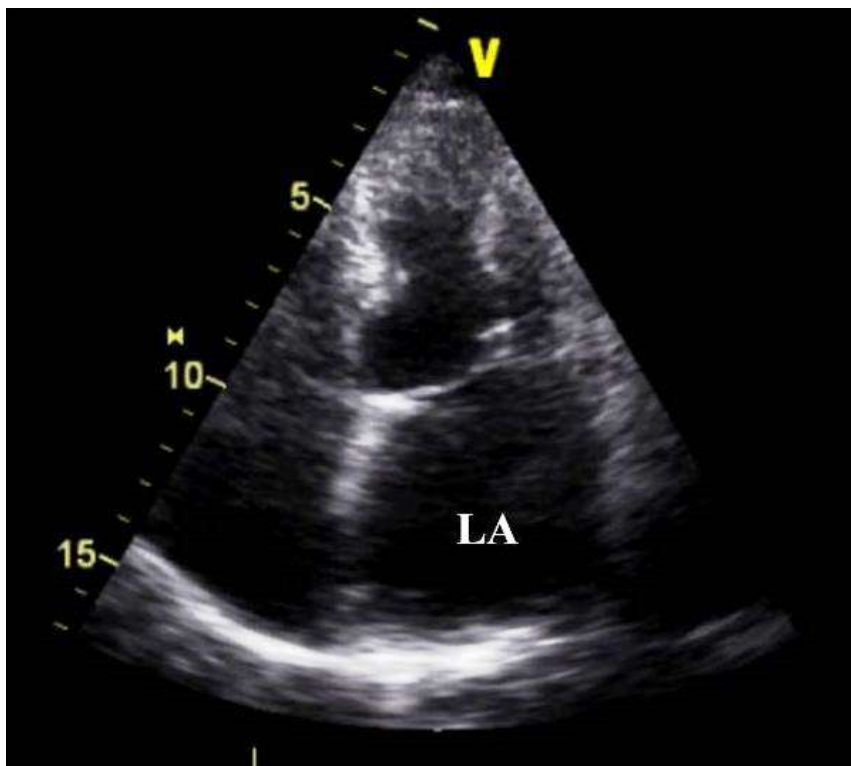


Figure 1. Transthoracic echocardiography displaying a left atrial (LA) dilation (courtesy of Rui Martins, MD, Cardiology Department).

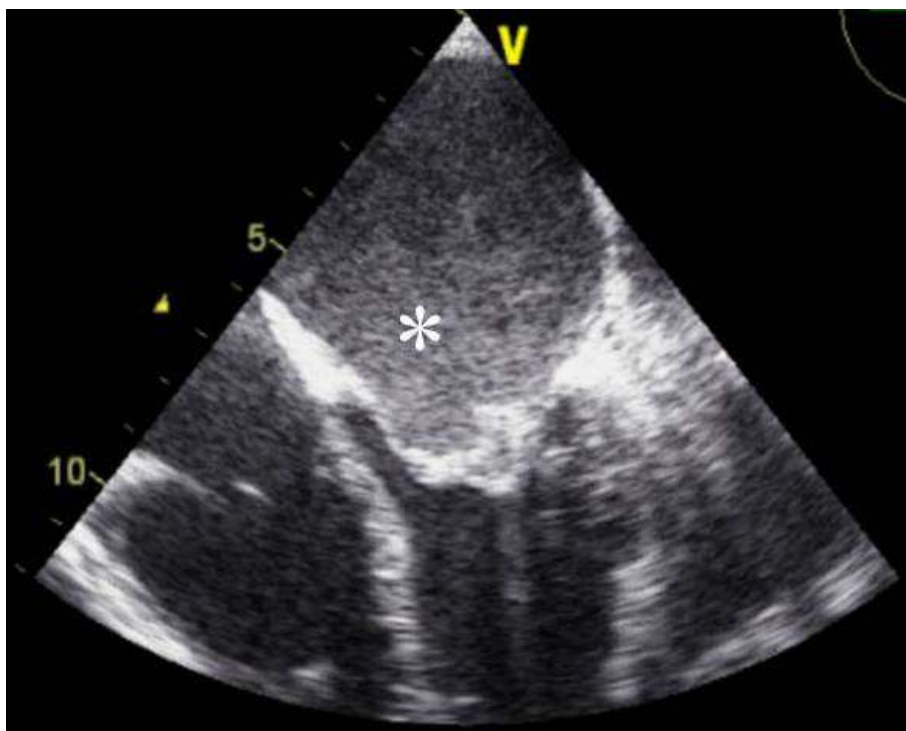


Figure 2. Left atrial spontaneous echocontrast finding (*) in transesophageal echocardiography (courtesy of Rui Martins, MD, Cardiology Department).

Multiple studies have demonstrated that the presence of LA and LAA SEC is a risk factor for the development of local thrombus [33]. Patients with nonvalvular atrial fibrillation with SEC have both, a significantly higher risk of developing stroke or other embolic events, and reduced survival [34].

The predilection for thrombus formation in the LAA has been well described and the ability of transesophageal echocardiography (TEE) to detect them has been established (35, 36). The pathogenesis of LAA thrombus formation is not fully elucidated but is likely to result from stagnation within the long, blind ended trabeculous pouch [37].

A reduced left ventricular ejection fraction was also suggested to be an independent predictor of PAF detection [16]. Other parameters have been proposed as quantitative surrogate parameters of elevated thromboembolic risk in patients with PAF. LAA function, measured by LAA ejection fraction and LAA flow velocity were impaired in patients with cardioembolic stroke caused by PAF, even if TEE was performed during a normal sinus rhythm [38].

D- Neuroimaging Findings

Noncontrast computed tomography is the most frequently diagnostic modality used in the evaluation of acute stroke in many hospitals, despite the widespread availability of MRI [39].

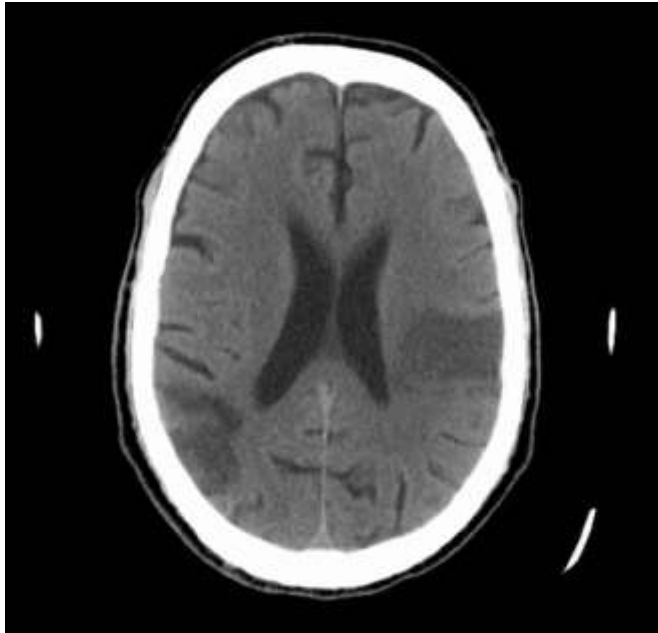


Figure 3. Cortico-subcortical ischemic dissemination in time and space: a previous cortico- subcortical infarct in the right hemisphere and a more recent one, on the other side (brain CT-scan).

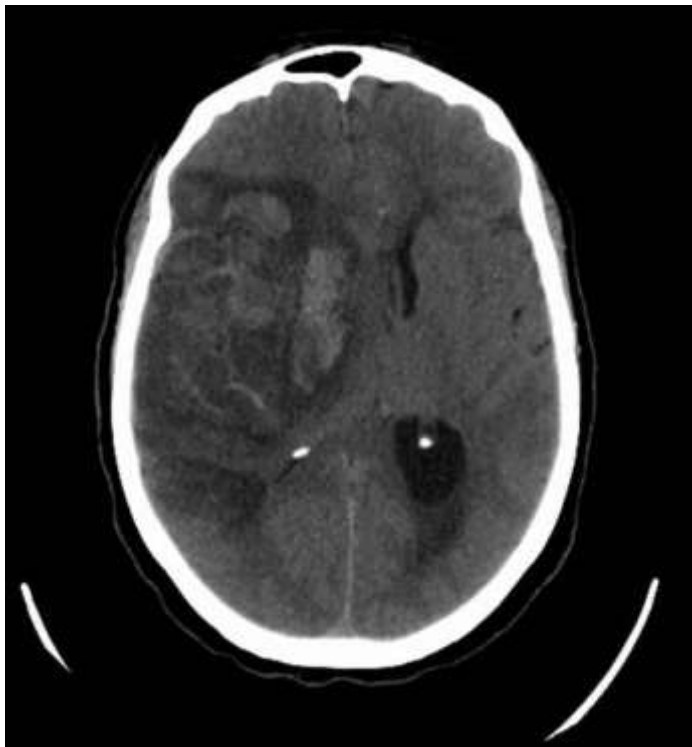


Figure 4. Brain CT-scan: hemorrhagic transformation of an ischemic lesion due to a right MCA occlusion.

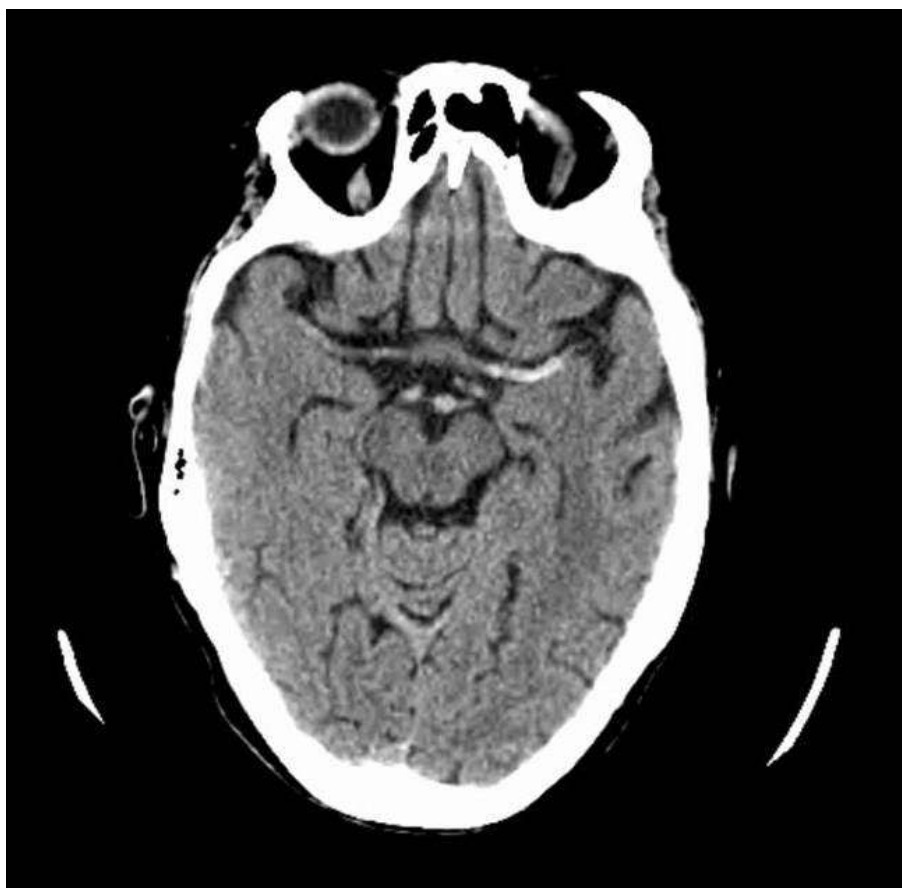


Figure 5. Brain CT-scan: Spontaneous hyperdensity in the M1 segment of the left middle cerebral artery.

Cardiac emboli travel through the intracranial circulation and often occlude middle-large size arteries. The identification of cortical involvement is characteristic of an embolic mechanism [5]. A distal cortical «wedge» appearance might be the pattern of infarction seen on CT scan or MRI [40]. An embolic event may also have a scattered pattern of infarction, that suggest an embolus that fractured and shattered into several pieces in the downstream vascular territory [23]. Single cortico-subcortical lesions or simultaneous strokes in different arterial territories, especially if bihemispheric, combining anterior and posterior circulation or concurrent systemic embolism are highly suggestive of cardioembolism [41-43].

The presence of previous cortico-subcortical infarcts in the same or other territories, detected during the evaluation of the most recent stroke is also highly suggestive of a cardiac source (Figure 3). Hemorrhagic transformation of infarction (HTI), usually asymptomatic, is a recognized natural consequence of cerebral infarction, occurring in up to one third of stroke patients [44]. Those who experience hemorrhagic transformation are more likely to have cardioembolic stroke [45] (Figure 4), particularly when embolism results in major arterial occlusion and failure of collateral flow [46].

It is plausible that delayed spontaneous recanalization achieved beyond 6-hour time window may increase the risk of hemorrhage in these patients [47]. Although unspecific, a

hyperdense artery sign on CT (Figure 5) can be seen in the presence of an acute intracranial embolus, a phenomenon that is well known and has been extensively documented.

Large middle cerebral artery (MCA) infarction is a frequent finding in patients with atrial fibrillation [48]. In the HAEST study [49] they represented 15% of all stroke patients with that rhythm disorder. In almost half of them a hyperdense middle cerebral artery sign (HMCAS) was noticed. It was recently suggested [50], by the histopatologic analisys of the thrombus, that HMCAS and the correspondent gradient-echo MRI blooming artifact (BA) may reflect the red blood cell content (RBC). In other words, those imagiological signs are present when the thrombus is RBC-dominant, and the absence of HMCAS or BA may indicate fibrin-predominant occlusive thrombi. Features that support the suspicion of cardioembolism due to PAF are summarized in Figure 6.



Figure 6. Ischemic stroke: clinical characteristics, imaging, electro and echocardiographic findings suggestive of a cardiac source of embolism due to PAF.

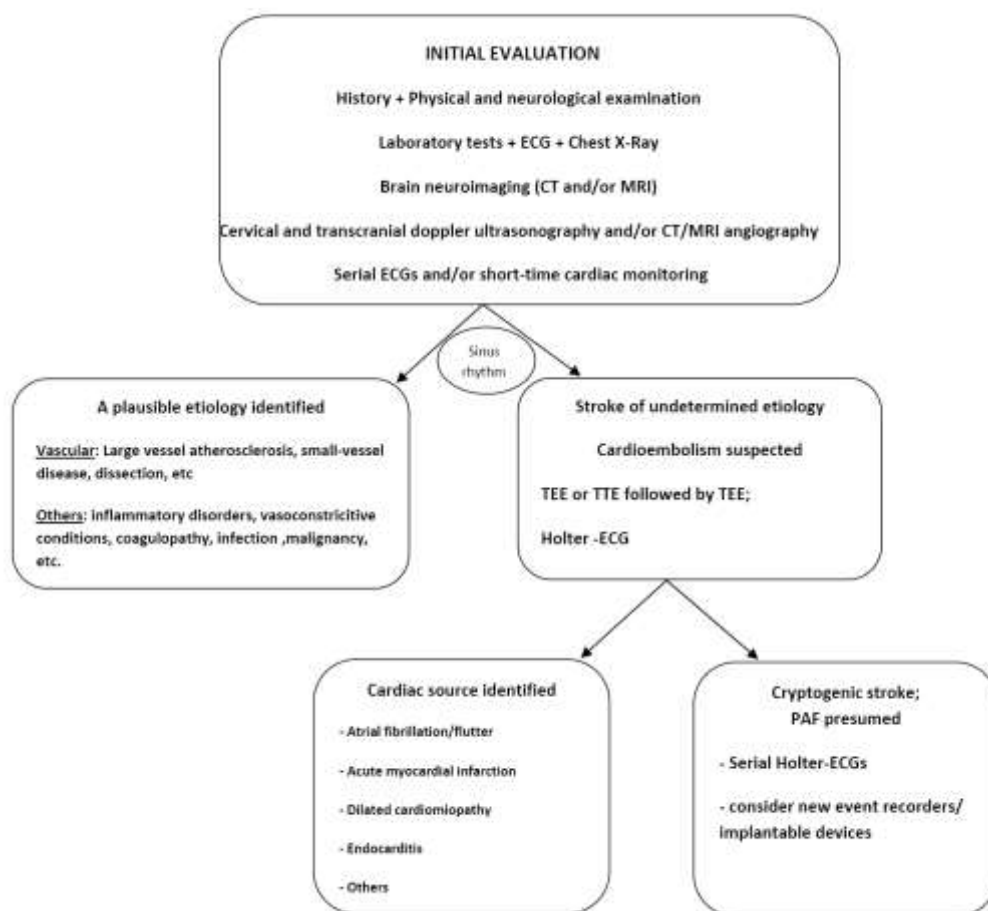


Figure 7. Stroke of unknown etiology : decision flowchart regarding cardioembolism.

CONCLUSION

Atrial fibrillation is an old arrhythmia involved in a recent growing epidemic [51], mainly due to its age-related incidence. Clinicians that treat stroke patients should be aware that cryptogenic stroke due to PAF is a frequent condition. There are important informations that can be transmitted by a detailed medical history, physical and neurological examination, cardiac work-up, ultrasonography and neuroimaging. There is a new era of new devices that can monitor cardiac rhythms for long periods. Should we use them in every patient who has a stroke of unknown cause? Probably no, we say. Instead of an aggressive investigation in every patient, we prefer a sequential approach (Figure 7) in order to select those that must be closely monitored after a complete but negative initial work-up.

A validated score that helps us to identify these patients is needed.

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Chapter 60

NOVEL ORAL ANTICOAGULANTS FOR STROKE PREVENTION IN ATRIAL FIBRILLATION

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ABSTRACT

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, affecting 1-2% of the general population. The prevalence of AF increases with age and with a growing aging population it is expected to double over the next five decades. AF poses a significant risk factor for stroke, currently conferring a five-fold risk, with one in five strokes attributed to this common arrhythmia. Furthermore, strokes relating to AF are likely to be more debilitating than non-AF related strokes. This causes increased morbidity and mortality and leads to increased physical and psychological distress for the affected patients and their families; as well as additional cost to the community. Preventing stroke in patients with AF is therefore of paramount importance. Over the last few decades the benefit of anticoagulation in patients with AF has been increasingly understood and gained popularity. This is usually achieved with oral vitamin K antagonists, most commonly warfarin. However, novel oral anticoagulants (NOACs) have advantages over warfarin and might provide an alternative to this therapy. This chapter discusses the NOACs dabigatran, rivaroxaban and apixaban and their evidence-based role in anticoagulation for patients with AF.

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, affecting 1-2% of the general population [1]. Its prevalence increases significantly with age and is expected to double in the next five decades, reaching ten million in the US by 2050 [2]. Among people younger than 50 years of age the prevalence of AF is less than 0.5%, while this increases by

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up to thirty-fold for people over 80 years old, reaching 5-15% [3]. Furthermore, other patient populations (e.g., those with a permanent pacemaker) have a higher prevalence, often in excess of 30-35% [4, 5].

Atrial fibrillation was once considered a “benign” arrhythmia [6] as it can be associated with mild or no symptoms. However, it is now well understood that, even in the absence of symptoms, if left untreated AF can cause significant morbidity and death [7]. It confers a doubling in death rate, a five-fold increase in the risk of stroke, increased hospital admissions, impaired exercise capacity, reduced quality of life and tachycardia-induced cardiomyopathy leading to heart failure.

WHY DO PATIENTS WITH AF HAVE A HIGHER RISK OF STROKE AND WHY IS THERE A NEED FOR ANTICOAGULATION?

The cause of strokes and transient ischemic attacks (TIA) in patients with AF was long believed to be thrombus formation in the left atrium and particularly in the left atrial appendage (LAA), with resulting fragmentation and dispersion in the arterial circulation. For up to 90% of patients with non-valvular AF in whom thrombus is identified, it is in the LAA [8]. With its long and tubular structure and a narrow junction with the atrium, the LAA is anatomically prone to hemodynamic stasis [9]. Coupled with its AF-associated dysfunction as a result of reduced peak emptying flow velocities, this is the major contributor for thrombus formation. It is now understood however that thrombus formation in the LAA is not the sole reason for stroke. Up to 25% of patients with high-risk AF could have other atherothrombotic mechanisms contributing to the increased stroke risk [10] making appropriate anticoagulation the most important treatment for reducing stroke and TIA risk in patients with AF.

WARFARIN VS. ANTIPLATELET AGENTS IN AF

Aspirin Use in Stroke Prevention in Patients with AF

With an increased understanding of the pathophysiology of stroke in AF in the last two decades, it has been appreciated that antiplatelet therapy alone is insufficient to significantly reduce the risk of stroke and TIA. Aspirin provides a 20% relative risk reduction in the incidence of stroke, [11] with a 0.5-1% risk of major bleeding [12]. Warfarin on the other hand offers a relative risk reduction of over 60% [11]. Compared to placebo this corresponds to an absolute benefit of 2.7% per year. In practice therefore, for every 37 patients with AF taking warfarin, one ischemic stroke is prevented annually. This also translates into a 26% relative risk reduction in all-cause mortality when compared to placebo [11], making warfarin an obvious recommendation for stroke prevention.

Dual Antiplatelet Therapy for Stroke Prevention in Patients with AF

The AF Clopidogrel Trial with Irbesartan for prevention of Vascular Events (ACTIVE) W study [13] investigated whether dual antiplatelet therapy with concurrent use of aspirin and clopidogrel provides the same benefit as warfarin in stroke prevention in patients with AF. Patients with AF and at least one risk factor for thromboembolic disease were randomized to receive either aspirin 75mg-100mg daily plus clopidogrel 75mg daily, or warfarin (aiming for a target International Normalized Ratio [INR] of 2.5). The combined endpoint of stroke, embolism, myocardial infarction (MI) and vascular death was reached in 5.6% of patients on aspirin and clopidogrel and 3.9% in patients taking warfarin (the relative risk ratio was 1.44, 95% confidence interval [CI]=1.18-1.76, $p=0.0003$), supporting the superiority of warfarin over dual antiplatelets and necessitating this trial to be halted early.

The risk of intracranial hemorrhage (the most problematic side-effect of warfarin) usually occurs with a supra-therapeutic INR ≥ 3.5) highlighting the importance of maintaining an appropriate anticoagulation level and regular monitoring. Achieving this can however be very difficult for some individuals who therefore spend a lot of time outside therapeutic range. Such individuals are at increased risk both from bleeding when the INR is too high and ischemic stroke when it is too low. The difficulty in establishing predictable and continuous anticoagulation remains the main drawback of using warfarin and an area where novel oral anticoagulants (NOACs) could prove to be superior.

THROMBOEMBOLIC RISK STRATIFICATION IN PATIENTS WITH AF

The anticoagulation practice for patients with AF has changed significantly over the last decade, particularly with the awareness that thromboembolic risk can vary significantly between individuals. The benefits of anticoagulation should therefore be weighed against the risk of thromboembolism before this is initiated. Various risk stratification scores have been used to predict thromboembolic risk in AF but the two most widely accepted are the established CHADS₂ and the more recently introduced CHA₂DS₂-VASc; where points are allocated according to the risk profile of each patient (Table 1).

The CHADS₂ score is a useful, simple and rapid scoring system for initial assessment of thromboembolic risk: patients can be categorized to low risk (score =0), medium risk (score =1) and severe risk (score ≥ 2). It has been validated by many studies and despite some recently recognized limitations, it still features in many international guidelines [1, 14-17]. The current guidance from the American College of Chest Physicians (ACCP) published in 2012 [15] for example recommends oral anticoagulation for patients at medium or high risk with CHADS₂ ≥ 1 . For patients in this group who are intolerant or unwilling to consider oral anticoagulation, dual therapy with aspirin and clopidogrel is recommended. This represents a change from the 2008 guidance [18] from the same college where anticoagulation was only recommended for patients with a CHADS₂ score ≥ 2 , acknowledging that even in this medium risk group (CHADS₂=1) the benefits of anticoagulation outweigh the associated risk.

However, further data have also emerged [19] on what were previously regarded as less well-validated risk factors for stroke, namely vascular disease, female gender and age

between 65 and 74 years. The CHA₂DS₂-VASc score incorporated these risk factors, allowing for an even more detailed and accurate estimated stratification of the annual risk of stroke (Table 2) for patients with AF [20]. Furthermore, the system can identify certain categories of patients (e.g., females or patients with peripheral vascular disease or coronary disease) who remain at increased risk of stroke but who would not have been identified using the CHADS₂ score alone.

Table 1. Shows how the two most commonly used stroke risk scoring systems can be calculated

CHADS ₂	Condition	Points allocated	CHA ₂ DS ₂ -VASc	Condition	Points allocated
C	Congestive heart failure or left ventricular (LV) dysfunction with ejection fraction (EF) ≤40%	1	C	Congestive heart failure or left ventricular (LV) dysfunction with ejection fraction (EF) ≤40%	1
H	Hypertension	1	H	Hypertension	1
A	Age ≥75	1	A ₂	Age ≥75	2
D	Diabetes	1	D	Diabetes	1
S ₂	Transient ischemic attack (TIA), stroke or other thromboembolism	2	S ₂	Transient ischemic attack (TIA), stroke or other thromboembolism	2
			V	Vascular disease (e.g., peripheral artery disease, myocardial infarction, aortic plaque)	1
			A	Age 65-74	1
			Sc	Sex category i.e., female	1

CHADS₂ has a maximum of six points and CHA₂DS₂-VASc a maximum of nine points, where more points equates to higher thromboembolic risk

Currently, in accordance with the European Society of Cardiology (ESC) guideline [1], any patient with a CHA₂DS₂-VASc score ≥2 should be considered for anticoagulation, as the 2.2% risk of stroke per annum is less than the risk associated with anticoagulation. Furthermore, anyone with a CHA₂DS₂-VASc score =1 should also be considered for either oral anticoagulation or aspirin therapy. The guidance suggests however that oral anticoagulation would be the preferred option even for these patients with a relatively low risk of stroke. Only for patients with the absolute minimum risk of stroke and a CHA₂DS₂-VASc score =0 is there no benefit from oral anticoagulation as the benefit of anticoagulation does not exceed its associated risk. For such patients either aspirin or no treatment at all could be offered in accordance with the ESC recommendation.

Table 2. Using the CHA₂DS₂-VASc score an annual adjusted risk score for stroke can be estimated, and the benefits of anticoagulation considered

CHA ₂ DS ₂ -VASc score	Risk (annual adjusted risk)
0	0%
1	1.3%
2	2.2%
3	3.2%
4	4.0%
5	6.7%
6	9.8%
7	9.6%
8	6.7%
9	15.2%

DISADVANTAGES OF ANTICOAGULATION WITH WARFARIN

The major disadvantage of warfarin stems from the fact that its dose, safety and effectiveness can be influenced by various pharmacogenetics such as the cytochrome P450 2C9 gene, the vitamin K epoxide reductase complex 1 gene, various vitamin K containing foods (kale, broccoli, onion, spinach, lettuce, parsley), alcohol and any other medications which can act as cytochrome P450 inducers or inhibitors. Warfarin can therefore lead to an unpredictable dose response, necessitating close monitoring both on initiation and during maintenance therapy. Furthermore, when the INR is outside the therapeutic range there is increased risk of both ischemic strokes (when INR <2.0) and hemorrhagic strokes (when INR >3.5). The INR therefore has to be monitored frequently, as even in patients with stable dosing a change in diet for example can lead to either sub-therapeutic or supra-therapeutic levels. It is therefore recommended that for stable patients INR should be monitored every 4-12 weeks to confirm therapeutic range [15, 21, 22].

Nevertheless, the regular and often unpredictable need for blood testing required with warfarin can make it inconvenient; discouraging some patients from agreeing to take it either due to personal preference (e.g., needle phobia, inability to commit to regular blood tests) or geographical complications (e.g., living in rural areas with no ability to visit a facility for blood taking on a regular basis).

THE IDEAL WARFARIN REPLACEMENT

Any new oral anticoagulant should be as safe and efficacious as warfarin but at the same time more convenient and cost-effective. Despite the disadvantages of warfarin these are at least known and include slow onset of action, unpredictable pharmacokinetics and many drug interactions requiring close monitoring. Warfarin does however have many advantages, hence its use for almost a century after its anticoagulant properties were first described in 1922 [23]. All the side-effects are known, it is efficacious (especially when in therapeutic range),

inexpensive and with a once daily dosing regime, allows for good adherence. Furthermore, in cases of bleeding there are many therapeutic options to minimize harm, ranging from vitamin K replacement (for minor bleeding requiring non-urgent reversal), to potent antidotes such as fresh frozen plasma and prothrombin complex concentrates when urgent reversal is required (for example bleeding in critical areas [e.g., intracranial] or major bleeding [e.g., polytrauma]). Finally, an unintentional benefit of warfarin is that the frequent INR monitoring allows the physician to ascertain adherence. This can be very beneficial, before scheduled electrical cardioversion for example, where serial INR measurements within therapeutic range can confirm adherence and minimize the risk of stroke during the cardioversion. A new anticoagulant should therefore ideally be better than warfarin– it must have a reversal agent and a mechanism for allowing physicians to ascertain adherence and there should not be any new disadvantages. It must also be at a price considered acceptable in the new era of cost-effective pharmacotherapy.

NOVEL ORAL ANTICOAGULANTS

There are currently three NOACs approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the reduction of stroke and systemic embolism in patients with AF: dabigatran etexilate, rivaroxaban and apixaban. The discussion in this chapter will be limited to these three approved agents, although others undergoing development and testing might enter clinical practice in the not too distant future.

Dabigatran Etexilate

Dabigatran etexilate is an oral prodrug rapidly converted by a serum esterase into dabigatran, a potent, direct, competitive inhibitor of thrombin. It has an absolute bioavailability of 6.5%, with 80% of the given dose excreted renally. Its serum half-life is 12 to 17 hours, therefore requiring twice daily administration, but it does not require regular monitoring (Table 3). It was the first NOAC to complete a phase III trial, the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial in patients with AF [24] and the first NOAC approved by both the FDA and EMA. In the trial, 18,113 patients met the criteria with non-valvular AF and at least one risk factor for stroke and systemic embolism from:

- previous stroke or TIA
- left ventricular ejection fraction $\leq 40\%$
- New York Heart Association class II or higher heart-failure symptoms within six months before screening, and aged at least 75 years or
- aged between 65 and 74 years with diabetes mellitus, hypertension, or coronary artery disease.

The study participants were randomized equally to receive either dose-adjusted warfarin (INR 2.0–3.0), dabigatran 110mg twice daily or dabigatran 150mg twice daily. This was a

non-inferiority study in which patients with renal failure and a creatinine clearance of $<30\text{ml/min}$ were excluded in view of concerns about the accumulation of dabigatran and increased bleeding risk [25]. The primary outcome was stroke or systemic embolism, occurring in 199 patients on warfarin (1.69% per annum), 182 patients on the 110mg twice daily dose of dabigatran (1.53% per annum, relative risk reduction of 9%, compared to warfarin, relative risk ratio 0.91, 95% CI =0.74-1.11; $p<0.001$ for inferiority) and 134 patients on the 150mg twice daily dabigatran dose (1.11% per annum, relative risk reduction 33% compared to warfarin, relative risk ratio 0.67, 95% CI =0.53-0.82; $p<0.001$ for superiority). Both dabigatran doses therefore proved non-inferior to warfarin with regards to stroke and systemic embolism. Rates of life-threatening bleeding, intracranial bleeding, and major or minor bleeding were significantly higher with warfarin (1.80%, 0.74%, and 18.15%, respectively) than with either the 110mg dose of dabigatran (1.22%, 0.23%, and 14.62%) or the 150mg dose of dabigatran (1.45%, 0.30%, and 16.42%). However, there was a significantly higher rate of major gastrointestinal bleeding with dabigatran 150mg compared with warfarin, which was not seen with the 110mg dose. One important association noted was that myocardial infarctions (MI) were less frequent with warfarin, at 0.53% per annum compared to 0.72% in the 110mg dabigatran dose and 0.74% in the 150mg dabigatran dose. The difference between warfarin and the higher dose dabigatran was significant ($p=0.048$). This was attributed to the beneficial effect of warfarin in reducing MI which dabigatran did not have. Finally, and importantly, there was no significant difference in overall mortality between warfarin and either dose of dabigatran (warfarin annual mortality =4.13%, dabigatran 110mg =3.75% and dabigatran 150mg =3.64%).

However, after the initial publication of the RE-LY trial results, the authors published more data that became available subsequently [26]. When the electrocardiograms of patients were retrospectively reviewed they found that 28 patients had suffered a silent MI. No such occurrences had been reported during the trial. The authors claimed that with this added data there was no longer a significant difference in rates of MI in the warfarin group and the dabigatran 150mg group. This finding should be interpreted with caution however, given the retrospective nature of data collection. A recent meta-analysis [27] has also shown dabigatran to actually increase the relative risk of MI by 33% compared to warfarin, enoxaparin or placebo for long-term use (relative risk =1.33; 95% CI, 1.03-1.72; $p=0.03$) although overall this only contributed only to a small absolute risk increase of 0.27% annually.

Analysing all the results of the RE-LY trial would suggest that the lower dose of 110mg dabigatran twice daily was associated with a similar number of total strokes as warfarin but with reduced bleeding, while the higher dose was associated with fewer strokes but a similar bleeding tendency. On the basis of this data the FDA only approved the 150mg twice daily dose dabigatran for use in the US as a novel agent conferring some reduction in stroke when compared to warfarin, whereas both doses have been approved by the EMA.

There are still some concerns about the use of dabigatran, particularly considering that despite the lower number of strokes for the 150mg dose and less bleeding for the 110mg dose, there was no significant difference in mortality. It is possible that the lack of reversing agent for dabigatran in an emergency is a factor. Haper and colleagues [28] reviewed patients who had bleeding while taking dabigatran and concluded that among other risk factors including advanced age, reduced renal function and low body weight, the lack of a reversing agent was a contributory factor to increased mortality. The reduction in stroke risk with the 150mg dose and bleeding with the 110mg dose should therefore be balanced against the inability of

managing an emergency optimally with a reversal agent. An argument could be made in favor of dabigatran, particularly the 150mg dose twice daily, that as it causes less hemorrhagic stroke than warfarin then perhaps avoidance of intracranial bleeding is more important than managing such bleeding optimally. However, both the advantages and disadvantages of dabigatran should be explained to the patients in order to enable them to make an informed decision for the treatment.

Rivaroxaban

Rivaroxaban was the first oral direct Factor Xa inhibitor to complete a phase III trial of stroke prevention in patients with non-valvular AF (the ROCKET-AF trial) [29] and the first agent from this class approved by the FDA and EMA for this indication. In this trial 14,264 patients at moderate to high risk of venous thromboembolism (CHADS score ≥ 2) were randomized using a double-blind, double-dummy approach with rivaroxaban 20 mg once daily or warfarin (with the dose regularly adjusted to maintain an INR of 2.0-3.0). Patients with decreased renal function (creatinine clearance 30-49 ml/min) received a reduced dose of 15mg rivaroxaban once daily. Patients with worse renal function were not included in the trial. Rivaroxaban has a very good oral bioavailability of over 80%, a rapid onset of action and it is 65% renally excreted with 35% excreted unchanged [30], again raising some concerns about toxicity in patients with significant renal impairment. The primary endpoint was again stroke and systemic embolism. Using an intention to treat analysis, 269 patients on rivaroxaban (2.1% per annum) and 306 patients on warfarin (2.4% per annum) fulfilled the primary endpoint giving a relative risk reduction of 12% for patients taking rivaroxaban compared with warfarin, a relative risk ratio of 0.88, 95% CI= 0.74-1.03, $p=0.001$ for non-inferiority and $p=0.12$ for superiority. Rivaroxaban and warfarin were very similar in terms of major bleeding, with 3.6% and 3.4% per annum, respectively. However, intracranial hemorrhage was less commonly observed with rivaroxaban (0.5% per annum) than with warfarin (0.7% per annum) and the relative risk reduction was 33% compared to warfarin (relative risk ratio 0.67, 95% CI =0.47-0.93; $p=0.02$). This was partly counterbalanced by an increase in major bleeding from the gastrointestinal site witnessed in the rivaroxaban group (224 events [3.2%] in the rivaroxaban arm compared with 152 events [2.2%] in the warfarin arm, $p<0.001$). Furthermore, this trial failed to prove a significant mortality benefit for the NOAC as there were 582 deaths in the rivaroxaban and 632 death in the warfarin group (4.5% and 4.9% per annum, respectively; relative risk ratio= 0.92; 95% CI =0.82 to 1.03; $p=0.15$) (Table 4), once again raising concerns about the lack of a reversing agent in an emergency.

This study was heavily criticised however because the mean percentage time that the patients taking warfarin had within the therapeutic range of INR was only 55% [31]. Although the authors claimed that 55% was an acceptable rate, many clinicians would consider this a relatively low percentage, particularly for patients enrolled in a trial, given that real-world experience both from North America [32,33] and Europe [34] shows that it is possible to maintain a therapeutic INR around 70% of the time. It has been argued therefore that this trial only succeeded in comparing rivaroxaban with poorly controlled warfarin.

Apixaban

Apixaban was the second direct oral factor Xa inhibitor to complete a direct head to head phase III trial with warfarin for stroke prevention in patients with AF (the apixaban versus warfarin in patients with AF [ARISTOTLE]) trial [35] and subsequently approved by both the FDA and EMA. Apixaban has rapid absorption, a shorter half-life of 12 hours and is 25% renally excreted (Table 3). Twice daily administration is therefore necessary but the added benefit of this is that in case of bleeding less time will be required for its anticoagulant effects to wear off. A total of 18,201 patients with AF and at least one of the following risk factors for thromboembolism were included:

- aged at least 75 years
- previous stroke, TIA, or systemic embolism
- symptomatic heart failure within the previous three months or a left ventricular ejection fraction of $\leq 40\%$
- diabetes mellitus or
- hypertension requiring pharmacologic treatment

With the use of a double-blind, double-dummy design, patients were randomized to either dose-adjusted warfarin, aiming for INR 2.0–3.0 or apixaban 5mg twice daily (or 2.5 mg twice daily in those who met two or more of the following criteria: age >80 years, body weight $<60\text{kg}$ or mild renal failure with creatinine $>1.5\text{mg/dL}$ (133mmol/L) (patients with significant renal failure and creatinine clearance $<25\text{ml/min}$ were excluded). The primary objective of this trial was to determine whether apixaban was non-inferior to warfarin in reducing the rate of stroke (ischemic or hemorrhagic) or systemic embolism among patients with AF and at least one other risk factor for stroke. The primary safety outcome was major bleeding. The primary endpoint of stroke or systemic embolism occurred in 212 patients in the apixaban group (1.27% per annum) and 265 patients in the warfarin group (1.60% per annum), a relative risk reduction of 21% compared to warfarin, relative risk ratio = 0.79, 95% CI = 0.66–0.95, $p < 0.001$ for non-inferiority and $p = 0.01$ for superiority. Both the rate of hemorrhagic and ischemic/undefined strokes were lower in the apixaban group (relative risk reduction by 49% in the former and 8% in the latter). Fatal or disabling stroke was more common in the warfarin group with 117 patients (0.71% per annum) being affected compared to 84 in the apixaban group (0.50% per annum), conferring a relative risk reduction of 29% when compared with warfarin. The ARISTOTLE trial is, to date, the only one that showed decreased mortality with a NOAC compared to warfarin (death from any cause was 3.52% in the apixaban group compared to 3.94% in the warfarin group, [relative risk reduction 11% compared to warfarin, relative risk ratio = 0.89, 95% CI = 0.80–0.99, $p = 0.047$]). However, for cardiac causes specifically (including death from hemorrhagic stroke), there was no significant difference (death was 1.80% per year in the apixaban group and 2.02% per year in the warfarin group [relative risk ratio = 0.89; 95% CI = 0.76–1.04]). Similarly, death from non-cardiovascular causes (including fatal bleeding other than hemorrhagic stroke) was 1.14% per year in the apixaban group and 1.22% per annum in the warfarin group (relative risk ratio = 0.93; 95% CI = 0.77–1.13) and the rate of MI, although lower in the apixaban group, did not reach statistical significance. Major bleeding however occurred in 327 patients in the

apixaban group (2.13% per annum), compared with 462 patients in the warfarin group (3.09% per year [relative risk reduction 31% compared with warfarin, relative risk ratio= 0.69; 95% CI =0.60-0.80; $p<0.001$]). The rate of intracranial hemorrhage was 0.33% per annum in the apixaban group and 0.80% per annum in the warfarin group (relative risk reduction 58% compared to warfarin, relative risk ratio= 0.42; 95% CI =0.30-0.58; $p < 0.001$) (Table 4). Although apixaban possibly showed the most promising results out of all the NOAC some concerns have also been raised regarding this trial; in particular, the time spent in therapeutic range when taking warfarin was perhaps lower than what can be achieved in other centers [36], again raising the concern that apixaban might simply be better when compared to poorly controlled warfarin.

When Warfarin is not an Option

Apixaban is the only novel agent to date that has been studied in patients who were unsuitable for warfarin therapy. In the apixaban in patients with Atrial Fibrillation (AVERROES) study [37], it was compared to the use of aspirin for stroke prophylaxis. Unsurprisingly, apixaban performed better in terms of reduced stroke rate with comparable mortality and major bleeding outcomes. There were 51 stroke or systemic embolism events (1.6% per annum) among patients taking apixaban and 113 (3.7% per annum) among those taking aspirin (a relative risk reduction of 55% compared to aspirin, with a relative risk reduction =0.45; 95%, CI = 0.32-0.62; $p<0.001$). Death rates were 3.5% per annum in the apixaban group and 4.4% per annum in the aspirin group (relative risk reduction 21% compared with aspirin, relative risk reduction =0.79; 95% CI =0.62-1.02; $p=0.07$). All data taken together suggest that apixaban is safer than aspirin for stroke prophylaxis in patients who cannot tolerate (or refuse) warfarin therapy.

Apixaban Use in Patients with a High Risk of Thromboembolism and Bleeding

Apixaban has also been to date the only NOAC comparing anticoagulation against warfarin for patients in AF categorized according to two thromboembolic risk scores (CHADS₂ and CHA₂DS₂VASc) and one bleeding score (HAS-BLED) [38]. In a sub-analysis of patient risk in the ARISTOTLE trial there was no statistical heterogeneity of response across the different risk categories. A possible explanation for this was that apixaban could benefit every patient equally, regardless of their thromboembolic and bleeding risk. Alternatively, as commented in an accompanying editorial, being a subanalysis of the ARISTOTLE trial, this was not sufficiently powered to confirm such a difference and advised therefore for the results to be interpreted with caution [39]. In addition, careful analysis also suggested that the numbers needed to treat (NNT) to prevent one stroke varied significantly from approximately 120 for those with CHADS₂ =1 and about 60 for those with CHADS₂ ≥ 3 , indicating that patients with a higher thromboembolic risk might benefit more from a NOAC and this could significantly affect cost-effectiveness.

Table 3. Showing the relative pharmacokinetics of the novel oral anticoagulants

	Dabigatran	Rivaroxaban	Apixaban
Target	Thrombin	Factor Xa	Factor Xa
Dose	Twice daily	Once daily	Twice daily
Half-life in hours	12-17	5-9 (but effect lasting for 24 hours)	12
Renal excretion	80%	25%	33%
Hepatic excretion	20%	75%	67%

ORAL ANTICOAGULATION AND BLEEDING RISK, CAN WE PREDICT THE INDIVIDUALS WHO WILL BLEED?

Unfortunately there are common risk factors for bleeding and thromboembolism. Hypertension, previous stroke and older age put patients both at risk of bleeding and thromboembolism and therefore the relative benefit of anticoagulation should be assessed against predicted bleeding risk for every individual. There are currently three bleeding scoring systems for use in patients with AF: HEMMORR(2)HAGES, ATRIA and HAS-BLED. The HAS-BLED is an easy scoring system to use; in a recent comparison it outperformed the other two, although the ability to predict bleeding was only modest [19]. In order to calculate a HAS-BLED score, a single point is added for hypertension, renal disease, liver disease, previous stroke, labile INR, age ≥ 65 , other medication predisposing to bleeding (e.g., aspirin or other non-steroidal anti-inflammatory drugs [NSAIDS]) and alcohol intake. A score of ≥ 3 represents a significant risk of bleeding. At present the most important reason for estimating the bleeding risk is not to prevent initiation of anticoagulation but to identify patients who might benefit from risk factor modification e.g., stopping aspirin if not required, introducing anti-hypertensive medication and support to reduce alcohol intake in order to minimise bleeding risk [40].

A recent analysis from Denmark assessed patients in AF on warfarin with both the CHADS₂ and CHA₂DS₂-VASc scoring systems and the risk of bleeding using the HAS-BLED system. The findings support the view that anticoagulation should be offered even in patients at the highest risk of bleeding (HAS-BLED ≥ 3), as paradoxically this is the group of patients who benefit the most (probably because they also have a significant thromboembolic risk) [41]. The same appears to be true for apixaban use in patients with AF [38] where the incidence of major bleeding was 3.46% for patients with HAS-BLED ≥ 3 and 2.25% for HAS-BLED=2 while intracranial bleeding was 0.23% and 0.38% respectively.

Based on the currently available evidence the decision to anticoagulate an individual patient with AF remains a nuanced one, balancing the Scylla of thromboembolism against the Charybdis of hemorrhage, both risks imperfectly predicted by even the best risk stratification scores. However, even if there is a high bleeding risk, following appropriate discussion with the patient, suitable candidates should be offered anticoagulation. Novel oral anticoagulants, particularly apixaban, might prove very cost-effective in this high risk subgroup of patients.

Table 4. Showing the outcomes of the trials of the three novel anticoagulants versus warfarin

	Stroke or systemic embolism/ annum	Major bleeding/ annum	Myocardial infarction/ annum	Intracranial bleeding/ annum	Hemorrhagic stroke/ annum	Mortality/ annum	INR within therapeutic range
RE-LY							64%
Dabigatran 110mg	1.53%	2.71% *	0.72%	0.23% *	0.12% *	3.75%	
Dabigatran 150mg	1.11% *	3.11%	0.74% *	0.30% *	0.10% *	3.64%	
Warfarin	1.69%	3.36%	0.53%	0.74%	0.38%	4.13%	
ROCKET-AF							55%
Rivaroxaban	2.1% *	3.6%	0.9%	0.5% *	0.3% *	4.5%	
Warfarin	2.4%	3.4%	1.1%	0.7%	0.4%	4.9%	
ARISTOTLE							62%
Apixaban	1.27% *	2.13% *	0.53%	0.33% *	0.24% *	3.52% *	
Warfarin	1.60%	3.09%	0.61%	0.80%	0.47%	3.94%	

The * indicates a statistically significant difference between the novel oral anticoagulant and warfarin.

CONCERNS ABOUT THE USE OF NOVEL ORAL ANTICOAGULANTS

The results of the trials comparing the three NOACs with warfarin for stroke prophylaxis in AF highlight several important limitations. Patients on warfarin did not achieve a good time in therapeutic range. In the dabigatran trial patients on warfarin had a mean time in therapeutic INR range of 64%; in the rivaroxaban trial the patients had a mean time of 55% and in the apixaban trial the mean time was 62%. We therefore cannot extrapolate the results to patients who have remained stable on warfarin and in therapeutic INR range for more than 70-75% of the time, which is more representative of real-life practice in North America and Europe [32-34]. Furthermore, these anticoagulants are relatively new. Rare but possibly significant side-effects might not have become fully apparent yet. Some concerns have already been raised regarding the lack of specific reversing agents for them, their use in moderate renal failure, underweight or overweight patients or even in elderly patients [28, 42]. There is also the risk of both undercoagulation and supracoagulation during the period of switching a patient from warfarin to one of the NOACs [28]. Formal guidance is awaited in order for this process to be completed safely. In addition, there is a need for vigilance and the collection of appropriate information on possible side-effects in order to ensure that the profile of NOACs remains a safe one. This is especially important considering the case of the “novel” anticoagulant, ximelatragan, where increased episodes of lethal hepatotoxicity were not fully appreciated until phase IV trials, promptly leading to its immediate withdrawal. Finally, in an era of financial concerns, new medication will have to prove their cost-effectiveness in addition to their safety and improved efficacy [43].

SO WHAT SHALL I DO DOCTOR?

Many patients nowadays are very well informed about their medical conditions and it is likely that based on online resources they will be aware of the possibility of taking a NOAC instead of warfarin for AF. Undoubtedly for some patients a NOAC might be more appropriate but the important question remains however whether a NOAC is more appropriate for every patient? The answer is probably not, at least not yet as demonstrated in the following scenarios with recommendations based on currently available evidence:

Case 1

A 78-year-old man with hypertension, diabetes and AF has been on warfarin for four years. He is stable with an INR in therapeutic range >80% of the time. He requires blood monitoring every 4-6 weeks and is happy with the necessary dietary restrictions. He is seen in clinic and enquires whether he should be taking a NOAC.

This is a common scenario, seen in most cardiology outpatient clinics. None of the main trials were powered to compare a NOAC with good warfarin control and we therefore do not have the evidence to suggest that switching to a NOAC for this particular patient will reduce either his risk of bleeding or thromboembolism, so the advice should be to continue with warfarin. Had his time in therapeutic range been <60% instead, then a NOAC could have been offered.

Case 2

A 45-year-old man with no risk factors for thromboembolism is scheduled to undergo electrical cardioversion.

One of the benefits of warfarin use is that it enables physicians to ascertain adherence. In this scenario confirming adherence and therapeutic INR in the weeks leading up to the cardioversion is crucial as it minimizes the risk of thrombus formation and the possibility of stroke following the cardioversion. Patients anticoagulated with warfarin often present for elective electrical cardioversion but are found to have sub-therapeutic INRs. It is only at this point that they admit/ remember that they have missed a warfarin dose. NOACs will not allow such an opportunity for monitoring, potentially leading to electrical cardioversion of patients who have not been adequately anticoagulated. Such patients should be managed with warfarin until a few weeks after the cardioversion and the need for anticoagulation addressed subsequently, where a NOAC might then be appropriate.

Case 3

An 88-year-old woman with AF, partially sighted with previous TIA, hypertension and peripheral vascular disease who lives alone in an area with no easy access to a clinic or community practitioner to monitor INR. Her time in therapeutic range for the last year was 40%. Two months ago she had significant bruises and her INR was found to be 6.4 and since then she has refused to take warfarin.

A patient who spends significant time outside therapeutic range is at risk both of significant bleeding when the INR is high and ischemic stroke when the INR is low. This woman has a $CHA_2DS_2-VASc = 7$ and an estimated annual risk of thromboembolism of about 10%. She will therefore benefit greatly from anticoagulation. Given the difficulties in maintaining warfarin within therapeutic range and the geographical difficulties associated with regular blood testing, it would appear that the risks of warfarin outweigh the potential benefits. Some patients, especially younger ones, might be able to self-monitor INR and warfarin dose at home; this appears to reduce some of the side-effects associated with warfarin [44]. However, antiplatelet therapy either with aspirin monotherapy or dual therapy with aspirin and clopidogrel will only reduce this patient's thromboembolic risk moderately and should not be recommended as a warfarin substitute. Therefore, in keeping with the findings of the AVERROES trial [37], apixaban 2.5mg twice daily would be the most appropriate NOAC to use.

Case 4

A 73-year-old woman with permanent AF for six years and a coronary stent in the LAD, inserted 15 months ago. She also has hypertension and gastroesophageal reflux. She

tolerated warfarin, aspirin and clopidogrel for 12 months and currently, following the advice from the interventional cardiologist, takes aspirin and warfarin. Her time in therapeutic INR is 60-70%.

With an aging population facing both coronary disease and AF, this scenario will become more common. A NOAC will not offer any protection from stent thrombosis and therefore this woman needs to continue with aspirin. The use of a NOAC plus aspirin might prove superior to warfarin plus aspirin but this has not been assessed in a trial. There have been some concerns about dabigatran and an increased risk of MI [27]. In this case, on the basis of current evidence, it might be appropriate to continue with aspirin and warfarin and in the future the patient might even be allowed to continue on warfarin monotherapy [45]. If however she refuses warfarin, it would be acceptable to consider a combination of aspirin and apixaban or aspirin and rivaroxaban although there is no direct evidence to support this at present.

Case 5

A 67-year-old man with hypertension, diabetes and asymptomatic permanent AF. He refuses to consider warfarin and has been on aspirin monotherapy for seven years.

The results of the AVERROES study [37] indicate that this patient (who refused to consider anticoagulation) will have prognostic benefit if he takes apixaban 5mg twice daily. Although not directly compared with aspirin, the other two NOACs, dabigatran in the RE-LY trial [24] and rivaroxaban in the ROCKET-AF trial [29], are also likely to benefit this patient as they compared favorably with warfarin in those studies. The most cost-effective NOAC should therefore be considered for this patient. If however, cost-effectiveness is similar across the three options, then apixaban is the one that has the most evidence to support its use in this case.

It is important to highlight that NOACs can only be used in patients with isolated non-valvular AF. They are not appropriate for patients with AF and an additional pathology e.g., a metallic heart valve. However, although a NOAC should not be offered routinely as a first-line treatment for anticoagulation in isolated AF, they can provide an excellent alternative to warfarin, particularly for patients with a low percentage time in therapeutic range (e.g., case 3 above). Physicians should therefore monitor time spent in therapeutic range during their clinic appointments as we now have the option of switching to something that is likely to be more effective than warfarin for such patients.

CONCLUSION

The NOACs offer a variety of therapeutic options for patients in AF who require thromboembolic prophylaxis and are likely to shape the future of anticoagulation. However, on the basis of the evidence available to-date, considering on the one hand the benefits, risks and convenience they offer and on the other, their cost-effectiveness in “real life” clinical practice [43], one cannot recommend any of the NOACs as panacea and first-line therapy for

every patient. Some patients, particularly those with high thromboembolic and bleeding risk, with poor INR control or refusing warfarin are probably the ones to benefit most from a NOAC. Over the next few years a NOAC should therefore be offered initially to these high-risk patient groups. Warfarin will probably remain in use for the majority of patients with AF for the next few years and is likely to celebrate its centurial birthday. However, with improved NOACs on the horizon, over the next decade warfarin is likely to lose its place as the most commonly used anticoagulant for AF.

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Chapter 61

STROKE IN ELDERLY

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ABSTRACT

Cerebrovascular diseases are one of the leading causes of mortality and disability, and they necessitate long-term care. Vascular dementia, on the other hand, is the second most frequent cause of dementia in the geriatric population.

The nihilistic approaches to stroke have been changed as a result of increasing availability of stroke units with trained staff and the recent improvements in acute stroke treatment.

However, the shorter treatment intervals decrease the amount of patients who can benefit from treatment.

In addition to patient based and treatment-focused approaches, the determination of preventable strokes via population based and preventative approaches will reduce the social and economical burden.

STROKE IS A GERIATRIC DISEASE

According to the WHO definition, stroke is a sign or symptom related to focal or general cerebral function loss, which does not have any other cause except for vascular reasons. It appears suddenly and lasts more than 24 hours [1]. The definition of cerebrovascular diseases, on the other hand, not only involves conditions such as embolism, thrombosis, or vascular laceration, but also conditions related to blood vessels such as atherosclerosis, hypertensive atherosclerotic changes, arthritis, abnormal enlargement of vessels and developmental malformations, all of which occur in the brain as a result of pathological changes.

Stroke prevalence is defined as the number of new strokes and stroke incidences seen in a specific population in a specific time period, and also the total number of old and new strokes seen again in a specific population in a specific time period. Stroke incidence must be seen as the total number of hospitalized, sudden and fatal cases, which can not be hospitalized. The

American Heart Association reported that in USA 7,000,000 people, who were older than 20, managed to survive after stroke. They also reported that each year almost 610,000 patients experienced a first stroke and almost 185,000 patients experienced recurrent stroke [2]. Stroke ratios, which are arranged according to age depending on the country of the study, the demographical characteristics of the population, and the methodological approaches used in the study, display a change of 1-3 cases per 1000 patients. While this ratio was found as 1.67 for white men and 1.38 for white women, it was reported that this ratio was 3.23 for black men and 2.60 for black women in the Northern Manhattan Study [3]. These ratios are usually similar to each other in white populations on which most of the reliable stroke incidence studies are done. On average, 2 new stroke incidences show up in every 1000 people. The proportion of people living in those populations, who had a previous stroke, is around 6 per 1000.

Since the 1950s, there is a decrease in stroke frequency. In the study of Rochester, Minnesota, it was stated that there was a 46% decrease in cerebral infarct and hemorrhage frequency between the years 1950-1952 and the years 1975-1979 [4]. Another study indicated that stroke frequency decreased 20% from 1968 to 1976 [5]. Contrarily, stroke incidence was found to be 17% higher in the years 1980-1984 compared to 1975-1977, in spite of the fact that there were improvements in the treatment of hypertension. However, this result was explained by the increased usage of CT, which facilitates the determination of silent stroke incidences [6].

Totally, in most industrialized countries, 10% of death cases occur because of stroke. Japan and China are the countries in which death ratios are mostly related to stroke. Furthermore, stroke is the leading cause of adult death in Japan [7]. In the USA, approximately 130,000 mortal cases are related to stroke, and stroke is seen as the leading cause of death in adults [8]. The range of stroke mortality changes 5-10% in 1000 per year. According to 2008 data in Turkey, on the other hand, the death ratio due to cerebrovascular diseases in the age group of 40-45 was found to be 4.5%, whereas it was found to be 9.4% in people older than 75 [9]. In the USA, it was declared that the death ratio related to stroke was 34.8% and the death ratio exactly caused by stroke was 19.4% between the years of 1998-2008 [10]. The data acquired by the National Hospital Discharge Survey supported the idea that the decrease in stroke mortality can be explained by the increase in survival ratios after stroke rather than the decrease of stroke mortality. Recent statistics, however, indicated that the decrease in stroke mortality reached its maximum level and the total number of strokes can start to increase again. According to the projections made by the AHA for 2030, stroke prevalence will increase 24.9% compared to 2010 and there will be 4 million more patients suffering from stroke in the USA [11].

To conclude, 30% of patients with stroke die in one year, up to 20% of them die in the very early phases. Additionally, 1/3 of patients who survived after stroke continue their lives needing care from others. The need of care from others is a burden to the population in terms of both economical and social aspects.

Another reason for the increased social burden due to stroke, can be vascular dementia. In recent years, there has been an increase in the prevalence of vascular dementia due to the increased life span and also due to the proportion of increased stroke survivors.. Stroke is the second most important cause of dementia in the world. In Japan, vascular dementia comprises 50% of all dementia cases, and stroke is known as the primary cause of dementia [12]. In western countries, on the other hand, vascular dementia comprises only 4-27% of all

dementia cases, and it is the most frequent dementia type after neurodegenerative dementias [13]. The geographical changes in prevalence probably depend on the geographical distribution of stroke, especially hemorrhagic stroke, and hypertension, all of which are seen more in Asia [14].

Although the prevalence of dementia was underestimated due to the lack of criterion in previous research, the prevalence of dementia after stroke was estimated as 20-25% in studies, which involved DSM-III-R criterion for the definition of dementia [15-16].

In one of the studies, excluding the cases of experienced stroke and dementia, it was found that the ratio for newly diagnosed dementias after stroke was 14% [17]. Another study determined that patients who had stroke had a 9 times greater risk for dementia 3 months after stroke, compared to a group of equally aged patients of the same gender, and predisposed to dementia [18].

Age and arterial hypertension are one of the most important risk factors for the development of dementia following stroke [19]. In one study, the ratio of the development of dementia one year after stroke was found as 5.4% for patients older than 60 and 10.4% for patients older than 90 [20]. The ratio of development of dementia following the development of lacunar infarct was 23.1% and this ratio was 4-12 times higher compared to control groups [21]. It should be noted that even the case of stroke is not clear; the dementia process can develop in multiple but silent cerebral infarct cases. The prevalence of silent cerebral infarct is 11% in the age group 55-64, while this ratio increases up to 43% in the age group older than 85 [22].

Risk factors are defined as the reasons for creating a tendency for a disease to be developed. Age is accepted as the most important risk factor and the most powerful indicator of stroke. After the age of 55, the prevalence of stroke doubles every ten years [23].

70% of people having stroke are older than 65 [24]. Stroke develops later in women compared to men. In the USA, the average age of stroke occurrence is 75 for women, whereas it is 71 for men [25].

In conclusion, stroke is one of the most important and frequently seen diseases in the geriatric population, leading to significant mortality and disability.

PREVENTABLE STROKE

In spite of the fact that there are social awareness studies and that recanalization-reperfusion strategies are thought of as the only effective treatment for patients with acute ischemic stroke, only a few patients having stroke can benefit from this prevention because the window for this option is highly limited. Via the widely spreading stroke units, with private staff members for stroke treatment and follow-up, the ratios for stroke mortality and disability decreased. In spite of the improvements in treatment and patient care, on the other hand, 1/3 of patients die each year [26]. That's why, while there is a search for methods to increase the thrombolytic treatments for candidate patients, public health must be arranged to treat processes related to stroke and particularly, to know how to prevent strokes.

As the main cause for long-term disability, stroke results in enormous problems for patients, families of patients, and health corporations in terms of social, economical, and emotional aspects of life. The estimated whole-life cost of a patient with stroke is 140,048

USD in the US, where one person dies of stroke every 4 minutes [27]. In recent years, although the improvements, especially in acute stroke treatment, are exciting because it results in a decrease in the helplessness of stroke; the number of patients who undergo and succeed with these treatments is not enough. Therefore, it is very important to take measures for both primary and secondary stroke prevention.

It is highly important in the definition of preventable stroke to diagnose and especially consider temporary ischemic attacks; it is also essential for society to investigate and find necessary treatments for the carotid artery and atrial fibrillation in specific age groups in terms of large vein atherosclerosis and cardioembolic strokes, which are the main causes of stroke. In addition, the aim to decrease the frequency of hypertension is another important point for society because it is a shared part of the etiopathogenesis of all different stroke groups. Such measures and efforts, taken to prevent primary and secondary stroke attacks, can be the most appropriate approaches in terms of social security politics.

The most important segment of the preventable stroke group is temporary ischemic attacks. As yet unburdening but alarming signs, transient ischemic attacks should be regarded as signals, which should not be missed, for both patients and clinicians.

TIA is defined as neurological dysfunctions related to focal cerebral ischemia, generally lasting less than one hour, and without the symptoms of acute infarct [28]. TIAs can be considered similar to stroke in terms of their similar etiopathogenesis and physiopathology. The symptoms must reflect dysfunctions in a specific area in the brain. Global definitions, such as confusion or loss of consciousness and symptoms such as seizures or involuntary movements derive from TIA [29]. A discriminative TIA definition can be made related to the duration.

The infarct findings in some patients' imaging after TIA claim that the definition of TIA is untrue; and those patients can be regarded as having cerebral infarct with transient symptoms [30]. It was reported that the incidences of TIA in a population older than 75 was 2.3/1000 [31]. When patients with stroke were analyzed in terms of TIA history, 4 studies in Europe claimed that 15-26% of strokes involved TIA before stroke and 43% of them had occurred in the last week [32].

50% of the infarcts following TIAs occur within one year, including 20% in the first month after TIA [33]. In another study, it was found that the risk of stroke after TIA occurs is 10% in 90 days, with half of them in the first 2 days [34]. 25% of patients with TIA suffered from stroke or another unwanted vascular experience within 90 days [35].

31-78.6% of TIA cases involve carotid artery stenosis and occlusion, whereas 16% of them include AF [36]. It is important to diagnose TIAs, which can be an indicator of future heavy strokes that are largely preventable with appropriate treatments. As a result, professionals should create suitable definitions about the symptoms related to the carotid system such as amorozis fugax, aphasia, paresis and hypoesthesia, as well as symptoms related to vertebrobasilar system such as vertigo, ataxia, diplopia, dysphasia in order to improve public awareness. Defining this clinical presentation, which is not different from stroke in terms of etiopathogenesis, with phrases like 'stroke precursor' instead of 'transient' may be more explanatory and may better emphasize its importance.

In terms of an etiology arranged according to the most common reasons, ischemic strokes can be mainly classified as atherothrombotic strokes, cardioembolic strokes and lacunar strokes.

Similarly, different results were also stated in the frequency of the subtypes of infarct according to the geographical areas of studies, characteristics of the chosen groups, and methods. While cardioembolic strokes as a source appeared in 15-30% of all infarcts, large vein atherosclerosis appeared in 15-40% and small vein lacunar infarcts appeared in 15-30% of the cases [37]. In spite of detailed investigations, idiopathic stroke cases were stated as 40%, which is a considerably high ratio [38].

LARGE VESSEL DISEASE

Approximately, 1/3 and 1/2 of all ischemic strokes are related to large vessel atherosclerosis. This type of ischemia occurs via the thrombosis resulted from the degeneration of the stabilization of atheroma plaques, which develop in large vessels especially in the areas of bifurcation. The developed atherothrombotic lesion not only results in stenosis and occlusion in the vessel but also makes it possible for an infarct to be developed in more distal watershed areas via hemodynamic mechanisms. Furthermore, it is possible for a distal vessel to be blocked by the pieces from an atherothrombotic lesion from artery to artery with the mechanism of embolism [39].

The clinical effects of atherosclerosis can only be seen in the middle-advanced ages, although it starts very early in adolescence and even in childhood ages. The presence of hypertension, hyperlipidemia and DM, smoking, low HDL, high LDL and homocysteine levels are known to facilitate the atherosclerotic process [40]. Atheromatous lesions may regress via various medications and diet, but they can be also observed in spontaneous regression. Atheromatos lesions develop very silently for years, but the complete embolism always occurs as a result of superimposed thrombosis. Usually, if the atheromatous process is heavy, then the possibility of a thrombotic complication is also high. However, these two processes do not always develop together. While a vessel of a patient with only a few atheroma plaques can be thrombosed, a thrombosed vessel can be absent in a patient with a clear atherosclerosis. It is both possible to see a patient have a vessel with different stenosis ratios together, and another patient to have an occluded carotid but not have carotid atherosclerosis. Poor dietary habits leading to obesity, the presence of familial history and infections and inflammations accelerating the atherothrombotic process may be indicators of the case. Studies related to the atherothrombotic process can lead to new results in clinic.

In the anterior circulation, atherothrombotic lesions are usually located at the origin of the internal carotid artery and siphon part, and in the posterior circulation, they are located at the origin and at the intracranial portion of the vertebral artery, as well as in the proximal basilar artery [41]. Stroke can be developed in different pathophysiological processes in patients with atherothrombosis in extracranial or intracranial vessels. When the thrombus is settled in an advanced atheroma plaque, the lumen either becomes narrowed or totally blocked. When the vessel is blocked, a blood clot is formed related to the ceased flow, it can go forward into the distal, and finally an infarct takes place if the collateral flow is not sufficient in the distal of blocked vein. Furthermore, the immigration of thrombus or plaque fragments into the distal can create an infarct in the distal via emboli from vein to vein. Additionally, cortical and deep watershed infarcts can occur in advanced stenosis or occlusions of extracranial or intracranial vessels due to brain hypoperfusion. Those different physiopathological processes can result in

different clinical reflections. There can be cases in which stroke takes place as a lone attack in a couple of hours or cases in which the neurological disease takes root with TIAs in a couple of days.

Carotid artery disease can be seen with other atherosclerotic processes in peripheral and coronary vessels. That's why, stroke attacks in patients whose other vessels are found to be malfunctioning, or patients who are in a specific age group with a high risk of venous diseases, or with TIA, may be prevented by non-invasive scanning methods such as Doppler.

The elimination of plaque in the internal carotid artery decreases the risk of stroke in the future. As an alternative method, surgery for the removal of narrowness came into being as a result of the insufficiency of medical treatments to prevent stroke in specific patients and the failure to prevent the development of atherosclerosis.

Endarterectomy operations are one of the most common operations used for almost 50 years. The 2-year risk of stroke in patients with bilateral carotid diseases was 69%, whereas the same risk was 22% in patients who were operated on [42]. Therefore, surgery can be advised to patients with symptomatic stenosis larger than 70% in institutions with operation complications lower than 6%. This benefit is also valid for aged people, and it is actually more clear for patients who are older than 75.

ASYMPTOMATIC CAROTID ARTERY DISEASE

In a population older than 65, more than 50% of asymptomatic carotid stenosis was determined in 7-10% of men and 5-7% of women [43]. In various studies, the risk of ipsilateral stroke was found as 1-3.4% in patients with 50-99% stenosis [44]. The risk is higher especially with rapid growth of plaques. The determination of silent infarcts via imaging in cases without having clinical symptoms yet is highly important. Evaluating the risk of stroke in those patients and determining the necessity of preventive treatment can be life saving.

When the studies of ACAS and ACST were taken together, this operation can be advised for patients having 60-99% asymptomatic carotid stenosis, with a life expectancy over 5 years, in institutions with a surgical risk lower than 3% [45].

Especially after the filter usage, the area of utilization of endovascular methods to eliminate advanced carotid disease in patients who were not appropriate for endarterectomy has expanded as a result of accumulated experiences and techniques developed related to the operation. There are many studies comparing carotid endarterectomy with the endovascular method in advanced narrowness of the carotid. Most of the findings of those studies showed that the endovascular method was not as successful as endarterectomy, especially in the prevention of stroke recurrence. However, it was observed that in patients who underwent stent angioplasty, most of the strokes seen, especially in early periods, were minor strokes and did not create any clinical problems [46]. As a randomized and head-to-head comparison study of endarterectomy and stent/angioplasty applications in carotid patients, CREST showed that, on the other hand, the clinical benefit of endovascular methods was not lower than endarterectomy in patients with angioplasty/stent especially younger than 70 [47]. The application areas of endovascular methods gradually increase in stenosis of vertebral artery proximal and intracranial stenosis, which cannot be reached via surgery. In the study of

SAMPRiSS, endovascular attempts for intracranial stenosis were not routinely advised because there were too many complications. However, it was still regarded as the only option for specific patients with intracranial narrowness whose conditions could not be stabilized via medical treatments [48]. As technical facilities and clinical experiences increase in the future, more areas for clinical application can be expected in patients with intracranial stenosis.

Intracranial artery stenosis is commonly seen in the basilar artery, V4 segment of the vertebral artery, MCA M1 segment, and intracranial ICA segments. They constitute 8-10% of all ischemic stroke cases [49]. In the study of WASID, the ipsilateral stroke ratio in symptomatic intracranial narrowness was found between 13-14% in 1.8 years [50]. The ratio for ischemic stroke was 19% in more than 70% of stenosis patients in one year [51].

CARDIOEMBOLIC STROKES

It is thought that 1/4 and 1/3 of all ischemic strokes are originated from cardioembolic reasons [52]. In contrast to the prodromal large vessel atherothrombotic strokes, emboli originated strokes are discriminated by clinically sudden onsets, maximal deficit in onset period with seizures, headache, and changes in consciousness. They can occur at any hour of the day and they do not have any stimulant episodes. The sudden onset of a stroke and the absence of prodromal signs indicates embolism in patients with a history of cardiovascular disease. Approximately equal in two hemispheres, the MCA territory especially in superior division territory is involved. Although large embolic blood clots can block large vessels, such as carotid, they usually hold onto areas in which lumen become narrow. An embolic blood clot may block the lumen where it is located or more often it breaks up into smaller pieces that lodge to smaller distal vessels. In this case, clinical effects can recover in a couple of hours. In the case of a sudden settlement of embolic blockage, on the other hand, collateral flow cannot develop and clinical tables settle down permanently. Embolic blockage of cerebral and cerebellar superficial branches can give rise to specific syndromes such as focal motor deficit, isolated aphasia or hemianopia. In 2 weeks after the first embolic event, an arterial or cardiac originated recurrent embolic event takes place with a 10-20% ratio [53]. Therefore, the emboli source should be determined in early stages.

The most common cause of embolic infarct is determined as chronic or new atrial fibrillation (AF). Nonvalvular AF is accepted as being responsible for 45% of cardioembolic strokes [54]. According to the Framingham Heart Study, patients with chronic atrial fibrillation experience 6 times more strokes compared to a population in the same age group with a normal heart rhythm. While the risk of stroke in the presence of AF is 1% in patients younger than 65, this ratio can increase to 8% in patients older than 75 with additional risk factors [55]. Literature shows that the first stroke in the presence of AF progressed 2 times more fatally compared to the cases without AF, and the risk of recurrent stroke in people who survived was higher [56].

AF is the most common rhythm dysfunction in patients with arrhythmia in USA [57]. The mean age of patients with AF is around 75, and 70% of them are in the 65-88 age group. 84% of the patients are older than 65 [58]. In two different studies with 5-15 years of observations, it was found that the prevalence of AF was between 5 and 9% in a population older than 60 [59].

The presence of incidental AF is a significant condition that deserves attention. 12% of patients who participated in Cardiovascular Health Study and 45% of patients in the SPAF 3 study were found to have incidental AF by coincidentally having EKGs for different reasons. [60-61]

The ratio of symptomatic AF to asymptomatic AF was found to be 12/1 [62]. The increased ratio of stroke in aged patients is not only dependent on the age-related increase in the ratio of AF; when the aged patients and young patients with AF were compared in terms of the risk of having a stroke, it was found that aged patients with AF had a higher risk for stroke. The presence of old age, volumetric increase in the left atrium and chronic rhythm dysfunction increase the risk in patients with AF. The atrial fibrillation itself is also thought to be a factor leading to a hypercoagulated condition. It was found that there was no difference between paroxysmal AF and chronic or permanent AF in terms of being a cause of stroke [63].

Most of the studies confirmed that warfarin is efficient for preventing stroke in patients with non-valvular atrial fibrillation. Those studies found that control groups had a 4.5% annual rate of stroke, while people who used anticoagulants had a 1.4% annual rate of stroke [64]. According to the common result acquired by primary prevention studies, oral anticoagulant treatments prevent stroke in 31 patients per 1000, and ASA prevents stroke in 15-20 patients per 1000 per year [65]. The efficiency of prophylactic anticoagulation is especially clear in patients older than 75 and having additional risk factors [66]. As a result, if the rhythm of patients with newly diagnosed AF cannot be ameliorated by cardio-version and antiarrhythmic medications, a prophylactic anticoagulant is advised. In the study of EAFT related to secondary prevention, it was determined that anticoagulant treatment was a quite effective treatment to reduce embolic complications in patients newly suffering from an ischemic attack. Also, ASA can be used in cases in which anticoagulant treatment was contraindicated and less efficient [67]. The ACTIVE-A study, on the other hand, showed that double antiaggregant usage was more effective in patients with AF compared to single aspirin usage, but it also produced an increase in hemorrhage complications [68]. 1/3 of adults with atrial fibrillation were people younger than 65 without additional risk factors, and it was found that this group, having a low risk of stroke, did not benefit from long-term prophylactic anticoagulation. Therefore, it is important for patients with AF to evaluate the risk of vascular incidents and the possible complications of using oral anticoagulants. For this reason, clinical scoring methods were developed and clinical reliabilities of those scores were controlled. CHADS2 and CHADS2Vasc scores are used in patients with AF in order to determine the vascular risk. While aspirin is preferred as prevention for patients with a low score, oral anticoagulant usage is advised for patients with moderate to high vascular risk. It is also possible to predict the annual hemorrhage ratios related to the use of oral anticoagulants via another clinical score called HAS BLED [69]. There are studies using not only clinical but also imaging methods to predict hemorrhage ratios. For instance, it is known that the determination of microhemorrhages in Gradient- Echo T2-weighted MR sequences increased the frequency of oral anticoagulants-dependent intracerebral hemorrhage [70].

The alternatives of warfarin were investigated for patients with AF because of its usage difficulties. It was found that ximelagatran, a direct inhibitor of thrombin, was as effective as warfarin in patients with AF in preventing ischemic stroke, and its hemorrhage complications were found to be less than warfarin [71]. Because it resulted in an increase in hepatic enzymes, on the other hand, ximelagatran could not be recommended for routine use. The

study of RELY performed in patients with AF, but without valvular heart disease, demonstrated that as a direct thrombin inhibitor, dabigatran was found to be as effective as warfarin in preventing vascular events and stroke, and to be slightly better in terms of hemorrhage complications [72]. Similarly, studies showed that apixaban and rivaroxaban, as direct thrombin inhibitors, can be used orally. They are an alternative to warfarin.

LACUNER STROKES

Lacunar strokes constitute the third group following large vessel atherosclerosis and cardioembolic strokes. While it was previously regarded to be in the same category with other ischemic strokes, its importance has become clearer in recent years. The clinical syndromes peculiar to this group of patients are easily recognized by clinicians and create positive expectancies about the prognosis. Major clinical syndromes are motor hemiparesis, pure sensory hemiparesis, dysarthria, clumsy hand, ataxic hemiparesis, and sensory motor syndrome [73]. It usually starts suddenly and recovery, sometimes almost the entire recovery, can take place in a couple of hours, days or weeks, even though the stroke was serious in the beginning.

Lacunar infarcts occur as a result of the occlusions of small penetrant terminal branches which do not have collaterals of large cerebral arteries [74]. Various mechanisms are proposed about the formation of lacunar infarcts. The increased arterial pressure produces gradual damage in the wall of the small penetrant artery, and vessel lumen becomes blocked via fibrosis connective tissue or fibrinoid material. It can end with lipohyalinosis pseudoaneurysm. Atheromatosis blocking the penetrant artery can create a lesion microatheroma. Atherosclerotic plaque in the large vessels can impair perfusion by blocking the penetrant artery orifice, or bringing about lacunar infarcts in embolies originated by the heart and large vessels [75].

Hypertension is the main component in etiopathology in terms of lacunar strokes. In almost every clinical and pathological investigation, a strong correlation was found between the lacunar state and chronic HT, and also a less strong but significant correlation between DM and HL. In the study of Rochester, Minnesota, hypertension was found in 81% of patients with lacunar infarct [76].

Population-based imaging studies show silent infarct as high as 28%, however lacunar infarcts were associated with a relatively good prognosis [77]. A potential cardioembolic source could be determined in approximately 8% (0-17%) of patients with lacunar infarct and more than 50% of stenosis and occlusion on the ipsilateral carotid artery in approximately 15% (3-63%) [78]. Another study showing 92% of determined lacunas are silent stated that those patients had unnoticed neurological deficits [79].

Small vessel dementia is called subcortical ischemic vascular dementia and it is regarded as the most common cause of dementia comprising almost half of the cases [80]. Both cortical and subcortical areas are influenced in lacunar events, while lesions are limited to subcortical areas in Biswanger disease. The ratio of the development of dementia 4 years after lacunar infarct was 23.1% and this ratio was 4-12 times higher than controls [21]. Dementia in lacunar infarcts is seen in 11% of patients at the end of the 3rd year, whereas this ratio increases up to 23% at the end of the 9th year [81]. The development of different kinds of

diagnosis criterion and the term ‘vascular cognitive impairment’ is used instead of ‘vascular dementia’ in order to show the causal relationship between the vascular process and cognitive impairment. This can be helpful in diagnosing the cases earlier and therefore include preventions for vascular dementia including subcortical damage and executive dysfunctions rather than memory impairments, especially in early phases. This is contrary to traditional dementia, which includes the development of deterioration in daily life activities and prioritized memory impairments.

Lacunar strokes should not be taken lightly, as innocent cases ending up with harmless results, because the accompanying diseases and especially recurrent lesions lead to cognitive deterioration.

Contrary to its 15-30% risk ratio in stroke, hypertension is the most important risk factor of intracerebral hemorrhages with very high mortality ratios of 25-60% [82]. In spite of the growing social awareness about hypertension, 72-81% of cases still have hypertension history [83]. The increase in prevalence of hypertension in aged people is the reason for the increased number of intracerebral hemorrhage in older adults. The cause of hemorrhage is impairment and rupture of the arterial wall as a result of hypertension. Arteriovenous anomaly and aneurysm are seen in people younger than 40, whereas the rupture of microangioma is seen between the ages of 40-70 [84]. The cause of recurrent lobar hematomas in normotensive people, older than 65, is cerebral amyloid angiopathy [85].

Hypertension should be a primary subject due to its part in the atherosclerotic process and its role in ethiopathogenesis of lacunar stroke and intracerebral hemorrhage. Meta-analyses of randomized controlled trials confirm an approximate 30-40% stroke risk reduction with the lowering of blood pressure [86]. Therefore, we should focus on the importance of not only the regular use of medications but also the regulation of dietary habits such as salt limitation and the prevention of a sedentary life style.

TREATMENT OF STROKE

Pathophysiology

Normal cerebral blood flow is 50 mL/100 g per minute. When an acute occlusion takes place in intracranial arteries, perfusion in cerebral tissue occupied in the distal of occlusion gets impaired and oxygen and glucose ratios decrease in brain parenchyma. If the perfusion pressure decreases too much, cerebral autoregulation becomes corrupted and cerebral blood flow becomes sensitive to the arterial blood pressure changes. If the cerebral blood flow decreases under 10 mL/100 g per minute, bioenergetic activities are rapidly lost, the membrane unity of neurons and surrounding glial tissue is lost, and the ion balance of the cell gets impaired. In the following process, sodium enters the cell, water inside the cell results in swelling of the cell, the calcium released by the internal calcium store is accumulated in the extracellular area and the accumulated calcium brings about the release of free radicals. Biochemical cascades are broken down via the released free radicals and necrosis of the cell occurs. In the case of a sharp decrease of cerebral blood flow, cell destruction can be seen via apoptosis without bioenergetic destruction. This area is called the ‘core’ tissue of the infarct and it is not possible to be saved with present treatments. When cerebral blood flow decreases

to levels between 10 mL/100 g/per minute and 20 mL/100 g/per minute, ion hemostasis and neuronal functions are lost, but the membrane units of the cells are preserved. The tissue in this area is regarded as recoverable tissue and is called penumbra. This area has recoverable tissue but cerebral blood flow must be quickly restored. In cases where cerebral blood flow decreases between 20 mL/100 g ile 50 mL/100 g per minute, neuronal functions are preserved. Cerebral tissue can tolerate these values for a long time without being destroyed, however, conditions such as hyperglycemia or hyperthermia can trigger the cell destruction. In an acute ischemic stroke, irreversible infarct tissues exist in the center and penumbra tissue, consisting of dizzy cerebral cells, in the periphery of this core area. The impairment of evoked potential waves and loss of neuronal functions in the penumbra area are shown, however, the penumbra area is accepted as a potential recoverable area via early recanalization. Beginning from the onset of the stroke, determining the core tissue and the recoverable tissue of the infarct is crucial for planning treatment strategies, and the ratio of the core/penumbra is a dynamic process. While core tissue increases from the beginning of stroke, recoverable tissues are exhausted in time [87].

THE TREATMENT OF ACUTE ISCHEMIC STROKE

The main purpose in acute stroke is to provide perfusion again. Intravenous thrombolytic treatment is one of the most important reperfusion strategies. It decreases death and disability. Intravenous thrombolysis is made by tissue plasminogen activator (tPA). The medication enables inactive plasminogen to be transformed into active plasmin. Plasmin is responsible for dissolving the fibrin plug. Intravenous thrombolytic treatment in patients with acute ischemic stroke was found to be reliable and effective both in randomized studies with controls and in international databases related to daily use. It has become the most important treatment strategy of recent years. Intravenous thrombolytic treatment remains important as the first choice in appropriate patients with acute ischemic stroke. If we define the reliability and efficiency criterion in pharmacokinetic terms, 1 in every 8 patients totally recovers, 1 in 3 patient partially recovers, and 1 in every 30 patients is harmed via this treatment. What is meant here by being harmed are the hemorrhagic complications and intracranial hemorrhages are the leading ones. It should be noted however, that all hemorrhages are not symptomatic and do not lead to death. After the efficiency of thrombolytic treatment was shown, studies related to lengthening the duration of the onset of treatment were planned in order to provide more patients with the benefits of the treatment. After the study of ECASS III, it was determined that intravenous thrombolytic treatment was efficient and reliable up to 4.5 hours. In this study, comparing the number of patients whose modified Rankin scores were between '0-1' and the group given the placebo, the number of patients in the rtPA group was found to be significantly higher. Even though intracranial and symptomatic hemorrhages were found to be higher in the treatment group, the death ratio appeared to be unreasonably higher in the placebo group. The results of the ECASS III study were immediately reflected to pilots, and the first 3 hours criterion was changed to 4.5 hours [88].

The recanalization rate with intravenous thrombolytic treatment is low, especially in cases with distal carotid T occlusion or proximal MCA occlusion with a high clot burden. From this point, endovascular treatment strategies were developed. In the study of PROACT,

investigating the efficiency of intra-arterial thrombolytic treatment following the first 6 hours after the symptom, it was demonstrated that pro-urokinase given as an intraarterial from a microcatheter provided 66% recanalization and significant increase compared to the placebo, with a ratio of 90 days of good prognosis. The efficacy of endovascular treatment was shown even if it was not approved by FDA. The studies of EMS, IMS I and II combining intravenous and intra-arterial treatments showed that a combined intravenous and intra-arterial treatment could also be reliable and efficient. Mechanical thrombectomy strategies were developed especially in cases resistant to intravenous treatment and contraindicated in pharmacological thrombolysis. Finally, almost 90% of the recanalization ratio was obtained via those mechanical approaches using retriever stent systems. The most important element for the achievement of a good clinical result is the case selection, regardless of the endovascular treatment approach [89].

Massive hemispheric brain infarcts such as malignant middle cerebral artery infarct have high death ratios (80%) due to different reasons such as cerebral edema, increased intracranial pressure, herniation and brainstem pressure. Decompressive hemicraniectomy surgeries are used for intervention of increased intracranial pressure irresponsive to conventional treatment methods in malignant middle cerebral artery infarcts and they have a life-saving effect on those conditions. Nevertheless, discussions about the appropriateness of surgical procedures and the criterions of patient selection are still not clarified. There are still unanswered questions remaining, such as the effect of the patient's age on the prognosis, the timing of surgery, the effect of hemispheric dominance, the size of the infarcted brain tissue and the monitorization of intracranial pressure. Although decompressive surgery was shown to lengthen the prognosis, the decision of decompressive hemicraniectomy for patients with massive brain infarct should be made depending on the patient.

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Chapter 62

TROPONIN AND ACUTE STROKE

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ABSTRACT

A number of studies and meta-analyses have indicated that elevations in serum troponin levels are seen in up to 20% of patients with acute stroke. There is some evidence to suggest that newer highly sensitive troponin T assays may detect more cases of elevated troponin after stroke. Elevated troponin has been suggested as an independent predictor of outcome following acute stroke and there is certainly an association with both mortality and dependent status following stroke. However, the value of troponin as a predictor has been questioned and it does not perform as well as simple clinical measures of stroke severity or lesion volume in predicting outcome. The question of what causes troponin elevation in stroke is still uncertain and may be multifactorial. It has been shown that this rise is rarely due to underlying ischaemic heart disease, renal failure or other known causes of elevated troponin levels. An association with ECG changes and atrial fibrillation has been demonstrated. Frank myocardial ischaemia appears to rarely be the cause as measured by CK-MB. Troponin levels are certainly associated with the severity of stroke and the brain lesion volume. There is also some evidence to suggest that elevated troponin levels are more common in anterior infarcts involving the cortex supplied by the middle cerebral artery, particularly the insular region. A syndrome of sympathomimetic overactivity arising from the cardunculus in the insular region has been suggested as a possible cause of elevated troponin levels after stroke. A similar mechanism has been proposed for contraction-band necrosis and takotsubo cardiomyopathy both of which can be precipitated by psychological stress and are associated with troponin elevations. It has been proposed that infarcts in either

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hemisphere may give rise to differential autonomic responses. However, to date the laterality of lesions associated with elevated troponin levels in acute stroke have been inconsistent. There remain a number of unanswered questions in the phenomenon of troponin elevations in stroke but this consistent finding demonstrates the close relationship between brain function and cardiac control. Further studies are required to delineate the likely causes of troponin elevations in individual cases and types of stroke, and to better understand the underlying pathophysiology. The final question remaining to be answered is whether or not any specific intervention might prevent or alleviate the presumed myocardial injury and lower any associated mortality and morbidity.

Keywords: Stroke, troponin, ischaemic, haemorrhagic, outcome, meta-analysis, review

1. INTRODUCTION

Worldwide, stroke is the second most common cause of death and accounts for 9% of all deaths [137]. Stroke is also a significant cause of morbidity and disability, being associated with the second highest impact in terms of disability-adjusted life years [132]. Whilst there is some evidence to suggest that in the developed world prevention strategies may be having a significant impact in reducing the incidence of stroke [53], this is likely to be more than offset by a well-documented ageing of these populations [132].

When assessing patients with acute stroke, accurate predictors of both short term and long term outcome would be of immense value in planning both acute treatment and longer term rehabilitation strategies. Clinical [140] and imaging data [61] have been found to be of relatively limited value in the acute setting, although repeat measures may be of more value. The finding of elevations of serum troponin, a marker of myocardial injury, in a small but significant proportion of patients presenting with acute stroke and the association of this elevation with a poor prognosis a little over 10 years ago [101] therefore held out great promise for a serological marker of stroke severity and outcome, available at the time of admission. The finding of elevations in serum troponin levels in acute stroke has also raised significant questions as to precisely what is the relationship between the mind (brain) and the heart in this setting. In this chapter we aim to distil the available evidence for the relationship of the mind and heart in the context of acute stroke and troponin levels.

Whilst a clear relationship exists between stroke and troponin, its nature is complex and the predictive value of elevations in troponin T and I is limited. What is more intriguing is the nature of the relationship and the light that this may shed on the aetiology of stroke and the factors which lead to a poor outcome following stroke. Thus, the overall purpose of this review is to critically analyse the available data with regards to factors associated with elevations in troponin levels in the acute stroke setting and look at the potential mechanisms involved with a view to further developing management strategies.

2. METHODS

2.1. Review of the Literature

A systematic search of databases (Cochrane, MEDLINE, PubMed) was conducted using the search terms: stroke; cerebral infarction; intracerebral haemorrhage; cerebrovascular accident and troponin. Searches were confined to the title and abstract. Only articles with abstracts in English were included. The initial search yielded 338 articles. Abstracts were reviewed and potential studies of relevance identified. Studies were only included if clear inclusion and exclusion criteria were given and raw data with respect to the frequency of serum elevations of troponin were provided. Studies were excluded if: subject recruitment was non-randomly selective (e.g., fourth generation troponin T negative cases excluded; 1 study) [108]; only cases with subarachnoid haemorrhage were included (8 studies) [57, 94, 124, 148, 181, 194, 205, 206]; or if raw data were not provided (4 studies) [28, 89, 174, 213]. Bibliographies of relevant studies and relevant reviews were screened to identify additional potential studies. Other references were identified from the authors' personal knowledge and review of relevant articles.

2.2. Statistical Analysis

For comparison of categorical data odds ratios (OR) with 95% confidence intervals (CI) were used and a χ^2 test was applied to give p-values where appropriate. For continuous variables, because in the majority of comparisons only means and standard deviations were available rather than raw data, a student's t-test for comparison of means was utilised. Whilst some of the measures compared were unlikely to have been normally distributed, because of the generally large sample sizes and lack of significant skew (as judged by medians) this compromise was felt to be acceptable. Where medians and a range were provided, a mean and standard deviation were estimated using standard methods [93]. P-values of < 0.05 were accepted as being statistically significant. No correction for multiple testing was applied.

For assessment of the predictive value of clinical features with regards to outcome, sensitivity and specificity with positive and negative predictive values and likelihood ratios were calculated using the Toronto University evidence-based medicine online statistics calculator [192]. This software also provides estimates for 95% CI.

The principal outcome measures identified were death and death or dependency, most commonly assessed using the modified Rankin scale [25]. Comparisons of outcome were performed using RevMan v5.2 [168]. An I^2 value of less than 25% was taken to indicate low heterogeneity suitable for analysis by fixed effects models otherwise random effects models were applied [88]. Comparisons of frequencies were similarly performed using generic inverse variance methods and entering frequencies and standard errors as natural logs. Funnel plots were used to assess for publication and other bias using standard error on the vertical axis which allows for plotting of 95% CI as straight lines [191]. For the meta-analysis of multivariate adjusted odds ratios natural log of the standard error was estimated by dividing the difference in the natural log of the quoted 95% CI by 3.92. Where confidence intervals were not provided standard error was estimated from a contingency table based on the quoted

adjusted odds ratio and the given frequencies of cases and controls, and the number of unfavourable outcomes.

3. TROPONINS

3.1. Troponin Structure, Function and Metabolism

The basic contractile apparatus of myocytes are the myofibrils [97]. Each myofibril is composed of thick and thin filaments. Thick filament is composed of myosin and the troponin protein complex is immobilized on the thin filament of the contractile apparatus of the muscle. [52]. The troponin protein complex consists of three subunits, namely troponin C, I and T [161]. The function of the troponin complex is to modulate contractile function of the sarcomere in response to cytosolic calcium. Thus, the troponin complex plays a critical role in the regulation of excitation–contraction coupling in the heart. Cardiac troponin C is identical to its skeletal isoform, does not have cardiac specificity and thus is not used in assays for the diagnosis of cardiac injury [197]. Troponin I is not expressed in skeletal muscle or other tissues during development [201] and is highly specific for myocardium. Monoclonal antibodies can distinguish the cardiac troponin T isoform from skeletal isoforms without cross-reactivity, allowing the conclusion that elevation of troponin T in serum originates from myocardium and always reflects myocardial injury of some form. Assays currently used to measure troponin T levels have cardiac specificity equivalent to that of assays for troponin I [196]. Troponins have cytosolic and structural pools. Release of troponin occurs first from the early appearing cytosolic pool and subsequently from the structural pool. Approximately 6–8% of intracellular cardiac troponin T has been reported to exist in a cytoplasmic form [117]. Troponins exhibit characteristic biphasic release kinetics following myocardial injury. Release from the cytoplasmic pool gives rise to peak serum concentrations 24–36 hours after injury, and structural protein release leads to a second blunted peak 2–4 days after injury. Troponin initially released from the cytosolic pool has a half-life in serum of about 2 hours. Continuing breakdown of the myofibrillary-bound complex of troponin and actin explains the prolonged elevation of serum troponin after myocardial injury [138]. Troponin remains increased in serum for 7±10 days [117].

Transient troponin release may be due to reversible forms of myocardial injury. Transient elevation in experimental models of vital exhaustion and in some exercise studies may be due to the release of troponin from the cytosolic pool only [151]. The failure of continuing release has been attributed to the lack of release of structurally bound troponin, commonly observed after irreversible injury to the myocardium [35].

3.2. Troponins As a Marker of Cardiac Ischaemia

Troponin is a highly sensitive biomarker of myocardial injury that is used in the diagnosis of acute myocardial infarction and for risk stratification of patients with acute coronary symptoms [115]. In 2000 a joint committee of the European Society of Cardiology and the

American College of Cardiology issued new criteria that acknowledged that elevations in biomarkers were fundamental to the diagnosis of acute myocardial infarction [7].

Troponin is very sensitive in the detection of myocardial injury from any underlying cause including acute myocardial infarction [199]. In most situations an elevated troponin level also indicates an adverse prognosis [82, 164]. Although troponin is highly sensitive for detection of cardiac injury, it lacks specificity for the diagnosis of myocardial infarction and can be elevated in a number of other conditions including sepsis [211], heart failure [47], renal failure [80], pulmonary embolism [70], tachyarrhythmias [180], cardiac trauma [3], pericarditis [26] and a number of other conditions [152]. Below is a review of some of the commoner pathologies other than acute coronary syndrome that can cause troponin elevation with an emphasis on those that may be relevant to stroke.

3.3. Troponins As a Marker of Other Pathologies

Measuring circulating troponin using highly sensitive assays has become the gold standard approach in diagnosing acute myocardial infarction. However, increased troponin levels are found in several other conditions.

3.2.1. Sepsis and Septic Shock

The cardiovascular system is frequently compromised by sepsis and always affected by septic shock. Although a number of mediators and pathways have been shown to be associated with myocardial depression in sepsis, the precise cause remains unclear. A high incidence of abnormal troponin levels in patients with sepsis (between 31%-85%) have been described, most in the absence of coronary artery disease on angiography [8, 16, 112, 188, 210]. There is some correlation of levels to the presence of left ventricular dysfunction as measured by echocardiography and a higher need for inotropic support [210]. There is also an association with lower survival rates [221]. The cause of troponin elevation in sepsis is probably multifactorial. It has been suggested that inflammatory cytokines released from neutrophils, particularly Tumour necrosis factor- α (TNF- α) and Interleukin-6 are responsible for direct myocardial depression and increased cell membrane permeability to troponin molecules in sepsis [225]. Decreased myocardial perfusion due to hypotension, increased oxygen consumption due to tachycardia, release of noradrenaline and adrenaline with subsequent vasoconstriction, and increased coagulation of the capillary bed have all been proposed as playing a significant part in myocyte damage and subsequent troponin release [112]. Cardiac toxins are another possible mechanism [109].

3.2.2. Pulmonary Embolism

For risk stratification of haemodynamically stable pulmonary embolism patients, current consensus guidelines of the American Heart Association recommend routine assessment of right ventricular function by cardiac biomarkers and echocardiography [100]. Serum troponins are elevated in up to 50% of patients with pulmonary embolism [222]. A positive troponin test is an important prognostic marker in pulmonary embolus, and predicts right ventricular dilatation and an increased number of segmental perfusion defects on lung scintigraphy [142]. These patients had higher risk of developing complications including in-

hospital death, prolonged hypotension, cardiogenic shock, inotropic support and mechanical ventilation. Moreover, after adjustment, an increased troponin T value was an independent predictor of 30 day mortality in pulmonary embolus. It has been suggested that troponin estimation may be used to help risk stratify patients with pulmonary embolus thereby allowing targeted use of more aggressive treatment [190]. In a study involving 64 normotensive patients with pulmonary embolism, troponin T was detected in 50% of patients [72]. All 8 in hospital deaths occurred in the troponin T positive group (OR 21; 95% CI 1.2 to 389). Increased troponin T was the only parameter predicting 15 in hospital adverse events (death, thrombolysis, cardiopulmonary resuscitation, inotropic support) [72]. Recently, pulmonary embolism has been reported as the most common non-acute coronary syndrome cause of increased troponin levels [99]. Release of troponin in pulmonary embolism is attributed to the combination of acute pressure overload within the right ventricle, impaired coronary artery flow, and the hypoxic state generated [70].

Pulmonary embolism is a recognised complication of stroke, but in general this does not occur until after a median delay of 20 days from when immobility is imposed at the onset of stroke [214]. Very occasionally, pulmonary embolism and stroke can occur simultaneously as a result of embolisation of a deep vein thrombosis to both the lungs and the brain through a patent foramen ovale [162].

3.2.3. Cardiac Failure

Troponin elevation is seen in up to 49% of patients with congestive cardiac failure [92] and is associated with an adverse outcome in terms of mortality and cardiovascular events [147]. There is a close correlation between troponin levels and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) levels in cardiac failure suggesting that the cause of troponin leak is due to cardiac strain [130]. Troponin levels are also useful in staging and prognostication for cardiac failure [6]. *In vitro* experiments with myocytes have demonstrated a link between myocardial wall stretching and programmed cell death [36]. Studies in isolated rat hearts have shown troponin degradation in response to increased preload independent of any ischaemia. Activation of the rennin-angiotensin and sympathetic systems may further compound the myocardial injury [109]. Congestive cardiac failure is an independent risk factor for stroke and higher mortality in the setting of stroke [102]. Cardioembolic stroke seems to be the most commonly associated mechanism, such that formal anticoagulation has been advocated [69]. Studies in acute stroke have demonstrated a clear association between the presence of cardiac failure and an elevated troponin, and an inverse correlation between troponin level and left ventricular ejection fraction (LVEF) [13]. NT-proBNP is also elevated in a significant number of acute stroke patients and is associated with a worse prognosis [169]. Whilst the evidence for a relationship is clear, what remains to be determined is which is chicken and which is egg?

3.2.4. Renal Failure

Cardiac troponins are frequently elevated in the absence of acute coronary syndrome among patients with varying degrees of renal failure, and troponin T is more frequently increased compared with troponin I in asymptomatic patients with end-stage renal disease [14]. Troponin T elevation is seen in approximately 53% of patients with renal failure compared with only 7% for Troponin I [62]. In addition, the prevalence of increased serum troponin T and troponin I increased with increasing severity of kidney disease. The

mechanisms for increases in troponin T concentrations in patients with kidney disease are not clear. There is emerging evidence that increases in troponin T in asymptomatic patients with end-stage renal disease indicates subclinical myocardial necrosis or injury [155]. There is robust evidence that troponin T is a powerful prognostic marker in end stage renal disease [49]. The reason for elevations in troponin levels in renal failure appear to be due to either an associated cardiomyopathy or silent coronary artery disease rather than any effect of impaired renal function on the clearance of troponins which are principally cleared via the reticuloendothelial system [62].

4. TROPONINS IN STROKE

A total of 29 observational studies in a variety of settings looking at the frequency of elevations in serum troponin levels with a variety of cut-offs and assays for both troponin T and troponin I were identified. Whilst many of the studies collected data prospectively, the core study design was essentially retrospective for all. A summary of included studies is provided in Table 1. Five previous meta-analyses or systematic reviews of earlier studies were identified [1, 104, 105, 110, 118] and an extension to these incorporating studies looking specifically at intracerebral haemorrhage and studies performed since 2009 is provided in Figure 1. The overall estimate for the frequency of serum elevations in troponin level for acute stroke was 10.85% (95% CI; 9.88 – 11.82). When analysis was restricted to studies which did not exclude known causes of troponin elevation the figure was 11.0% (95% CI; 9.18 – 13.22). The I^2 figure for heterogeneity was 0% and therefore the use of a fixed model was appropriate. However, there was wide variation depending upon the type of troponin measured, the cut-off used and the types of stroke included. The type of stroke has a significant effect upon the frequency of troponin elevation, with haemorrhagic stroke being more commonly associated. Relevant data are summarised in Table 2 and indicate that elevations in serum troponin levels are almost twice as common in haemorrhagic stroke than ischaemic stroke. The frequency of troponin elevation in the studies where both haemorrhagic and ischaemic stroke were included was closer to the figure for haemorrhagic stroke than ischaemic stroke which is surprising given the relatively low frequency of haemorrhagic stroke in these studies. There was some evidence for publication bias in the studies of ischaemic stroke (Figure 2). For studies of haemorrhagic stroke and all stroke there may also be some evidence of bias but the smaller number of studies and a lack of larger studies makes it difficult to draw any firm conclusions. However, the overall effect estimate was representative of the largest studies reported suggesting that despite possible publication bias these overall estimates of troponin elevation frequency in stroke are accurate. There was no clear pattern of change in the frequency of troponin elevation in stroke over time, if anything there appears to have been a fall in the frequency of troponin elevations over time, perhaps reflecting an increase in specificity with improvements in the assays used (Figure 3). However, this trend is not significant. Similarly for both troponin T and troponin I the cut-off used does not appear to show any consistent trend in the frequency of finding an elevated level (Figure 4).

Table 1. Details of studies included in meta-analysis

Author [ref]	Year	Country	Setting	Troponin		Stroke type	Exclusions	Collection time(s)	Follow Up	N	Outcome
				Type	Cut-off (µg/L)						
James et al.* [101]	2000	New Zealand	General wards	T	> 0.1	IS	None	12 - 72 Hrs	Discharge	181	Death
Dixit et al. [51]	2000	USA	Academic centre	I	≥ 0.4	IS & HS	None	Adm + Days 1 - 5	1 year	40	Death
Troøyen et al.* [203]	2001	Norway	Stroke unit	I	> 0.4	IS & HS	None	Adm + Day 1	Discharge	149	Death
Ay et al* [20]	2002	USA	Academic centre	T	> 0.1	IS	IHD	Days 1 - 5	ns	32	Brain infarction
Guerrero-Peral et al.* [77]	2002	Spain	Neurology unit	T	> 0.1	IS & HS	None	< 96 Hrs	Discharge	42	Death
Christensen et al.* [41]	2004	Denmark	Stroke unit	I	> 0.1	IS & HS	None	Adm	3 months	155	Death or dependence
Apak et al.* [13]	2005	Turkey	Neurological intensive care	T	> 0.1	IS & HS	IHD/CCF/AF/RF	< 24 Hrs	ns	62	Myocardial injury
Atam et al.* [18]	2005	India	Young stroke in hospital	T	> 0.1	IS & HS	None	12 - 24 Hrs	Discharge	40	Death
Di Angelantonio et al.* [48]	2005	Italy	Stroke unit	I	≥ 0.1	IS	None	Adm	Discharge	330	Death
Etgen et al.* [58]	2005	Germany	Neurology unit	T	> 0.03	IS	AMI	< 12 Hrs + Days 1 & 2	90 Days	174	Death or dependence
Maliszewska et al.* [135]	2005	Poland	Neurology unit	I	> 0.1	IS	None	ns	6 months	196	Death

Author [ref]	Year	Country	Setting	Troponin		Stroke type	Exclusions	Collection time(s)	Follow Up	N	Outcome
				Type	Cut-off (µg/L)						
Ay et al.* [21]	2006	USA	First stroke	T	≥ 0.1	IS	None	< 72 Hrs	ns	730	Cardiac enzymes
Fure et al.* [65]	2006	Norway	Stroke unit	T	> 0.04	IS	IHD	Adm & Day 1	Discharge	279	Death or dependence
Hays & Diring [86]	2006	USA	Neurosurgical intensive care	I	> 0.1	HS	AMI/UA	< 24 Hrs	Discharge	235	Death or dependence
Iltumur et al.* [98]	2006	Turkey	First stroke in neurological intensive care	I	> 0.1	IS	IHD/CCF/MI/CVD/RF/HF	Adm + Days 3, 5 & 10	ns	57	NT-proBNP levels
Maramatton et al. [136]	2006	USA	Neurosurgical intensive care	T	> 0.035	HS	AMI	Adm + Day 2	60 Days	49	Death
Barber et al.* [22]	2007	UK	Stroke unit	I	> 0.2	IS	None	6-24 Hrs	30 Days	222	Death or dependence
Jensen et al.* [107]	2007	Denmark	Neurology unit	T	> 0.03	IS	IHD/AF	Adm + Days 1 - 5	Mean 19 months	244	Death
Sandhu et al. [173]	2008	USA	Neurology unit	I	> 0.4	IS & HS	AMI/CCF/PE/RF	< 24 Hrs	Discharge	255	Death
Song et al. [187]	2008	Korea	Stroke unit	T	> 0.01	IS	IHD/CCF/RF	< 3 days	30 Days	416	Death or dependence
Garrett et al. [67]	2010	USA	Neurosurgical intensive care	I	≥ 0.4	HS	None	Adm + Days 1 & 2	Discharge	100	Death or dependence
Ghali et al. [68]	2010	Australia	Stroke unit	T	> 0.1	IS & HS	None	Adm +48 Hrs	Discharge	109	Death or dependence
Wira et al. [215]	2011	USA	Emergency department	I	>0.04	IS	CP	<24 Hrs	Discharge	161	Death
Beaulieu-Boire et al.[24]	2012	Canada	Neurology unit	I	> 0.03	IS & TIA	AF/RF	< 24 Hrs	90 Days	278	Death or dependence

Table 1. (Continued)

Author [ref]	Year	Country	Setting	Troponin		Stroke type	Exclusions	Collection time(s)	Follow Up	N	Outcome
				Type	Cut-off (µg/L)						
Darki et al. [45]	2012	USA	Neurology unit	I	≥ 0.1	IS	None	Adm	ns	137	Akinetic wall on echo
Furtner et al. [66]	2012	Austria	Stroke unit	hs-T	> 0.005	IS	None	Adm	90 Days	60	Death or dependence
Hajdinjak et al. [81]	2012	Slovenia	Pre-hospital admission	T	> 0.04	IS	Severe IHD/ RF	Pre-adm	Discharge	106	Death
Hasegawa et al. [85]	2012	USA	Neurology, neurosurgery and general wards	T & I	> 1.5 (I) > 0.1 (T)	HS	None	< 48 Hrs	90 Days	206	Death
Scheitz et al. [178]	2012	Germany	Neurology unit	T	> 0.03	IS	None	ns	Discharge	715	Death or dependence

IS = ischaemic stroke; HS = haemorrhagic stroke; TIA = transient ischaemic attack; IHD = ischaemic heart disease;

CCF = congestive cardiac failure; AF = atrial fibrillation; RF= renal failure; AMI = acute myocardial infarction;

UA = unstable angina; CVD = cardiac valve disease; HF = hepatic failure; CP = chest pain; Adm = Admission, ns = not stated

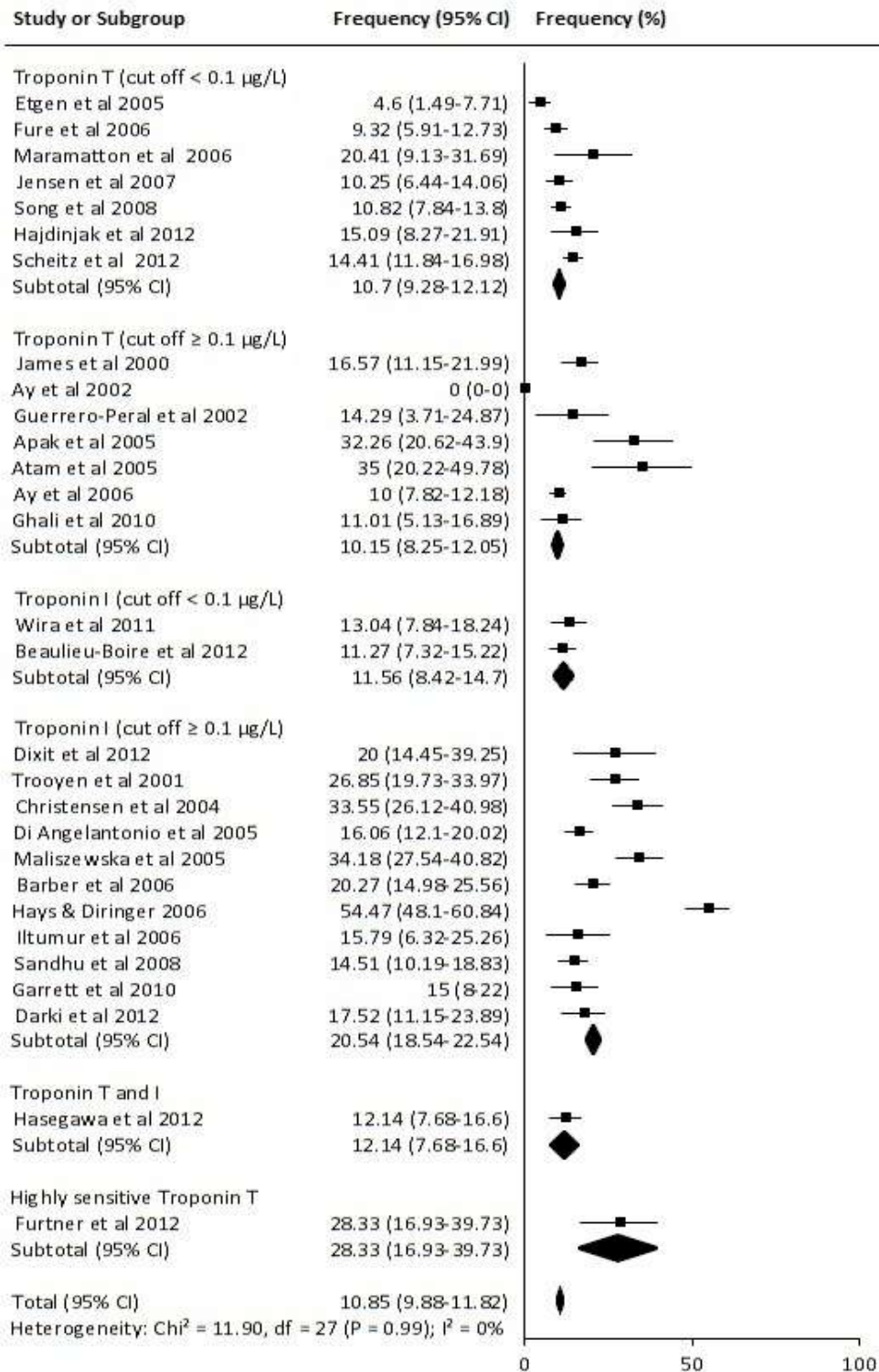


Figure 1. Meta-analysis of studies of frequency of serum troponin elevation in stroke.

However, it should be noted that several generations of troponin assays have been used across these studies and the particular tests used also varied. The frequency of elevated troponin I levels were in general higher than troponin T. It has also been demonstrated that troponin I levels in patients with acute ischaemic stroke are elevated when compared to age and gender-matched healthy controls [98].

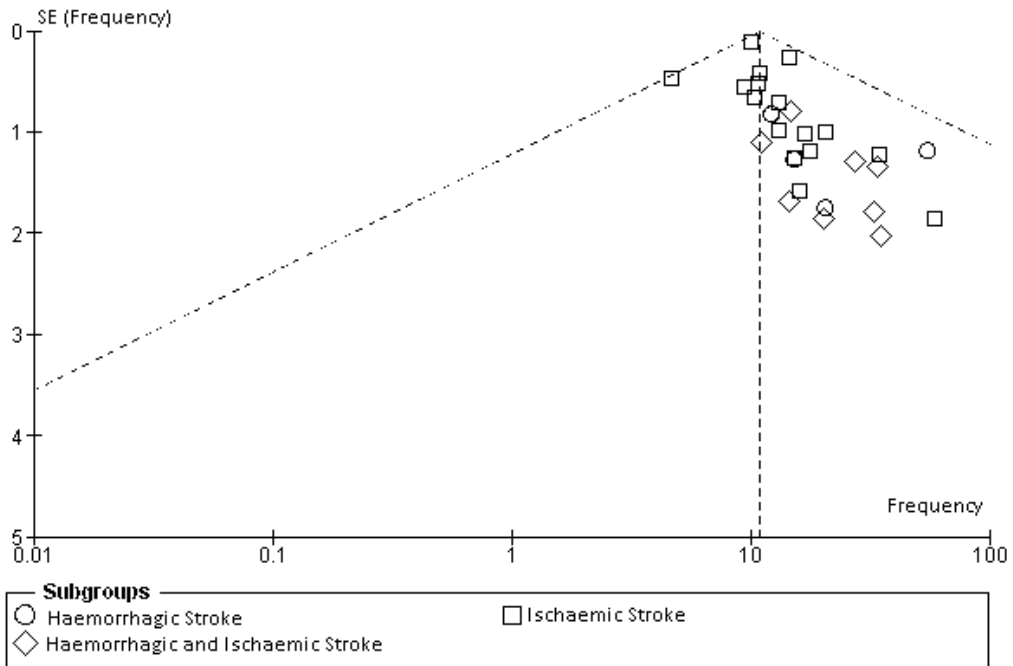
4.1. Clinical Associations of Troponin Elevation in Acute Stroke

Table 3 shows the collected data, where this was available, from all of the studies analysed with regards to clinical features evident at initial assessment. This included demographic data, past history, ECG assessment, neurological assessment and imaging data. In addition to the type of stroke being associated with elevations of serum troponin as discussed above, age is also related. Patients with acute stroke and a troponin rise are generally older. In this univariate analysis evidence of myocardial ischaemia on ECG or atrial fibrillation (prior to or on presentation) was associated with troponin elevation. As has been noted previously congestive cardiac failure and renal failure were both associated. Suspected cardioembolic aetiology and involvement of the insular cortex were also statistically significant. Interestingly, crude measures of stroke severity such as the Glasgow coma scale and other measures of level of consciousness were not significant, but the numbers were small. Conversely, the National Institutes of Health Stroke Scale (NIHSS) and the Scandinavian Stroke Scale (SSS) scores were both significantly associated.

Table 2. Frequency of troponin elevation in different stroke types

Type of stroke	Frequency of Troponin Rise		P-value
	N	(%)	
Haemorrhagic	590	(18.84)	
Combined	852	(18.54)	
Ischaemic	4320	(10.46)	<0.0001

The association with ischaemic features on ECG is to be expected and an increased frequency of such changes has been reported previously in the setting of acute stroke [29]. What is more difficult to determine is the cause of these changes. Acute myocardial infarction (AMI) appears to be an infrequent cause of troponin elevation but the diagnosis of AMI in this setting is not easy. Many studies excluded AMI and the criteria utilised to diagnose AMI were generally a combination of clinical symptoms, evolving ECG changes and serological markers. Only a few studies systematically looked for AMI and even then the frequency in subjects with elevations of troponin serum levels was only 6%. Interestingly, the frequency of AMI in the troponin negative group was 4% although many studies included AMI within the previous 2 weeks. What is clear is that frank myocardial infarction is an uncommon cause of troponin elevation in acute stroke. Lesser degrees of ischaemic changes on ECG may relate to prior ischaemic heart disease, current acute coronary syndromes and cardiac strain caused by arrhythmias or failure. There is clear evidence from the available data for all of these factors. However, even after excluding cases of renal failure the cause for troponin rise in acute stroke remains uncertain in the majority of cases [21].



SE = standard error.

Figure 2. Funnel plot for studies of troponin elevation frequency in stroke.

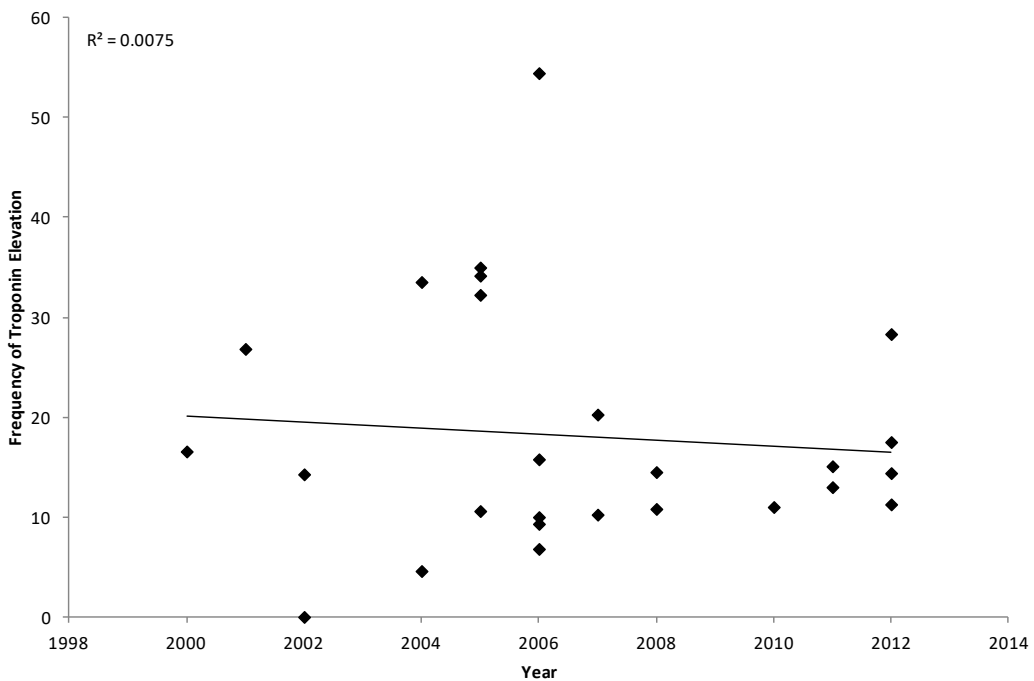
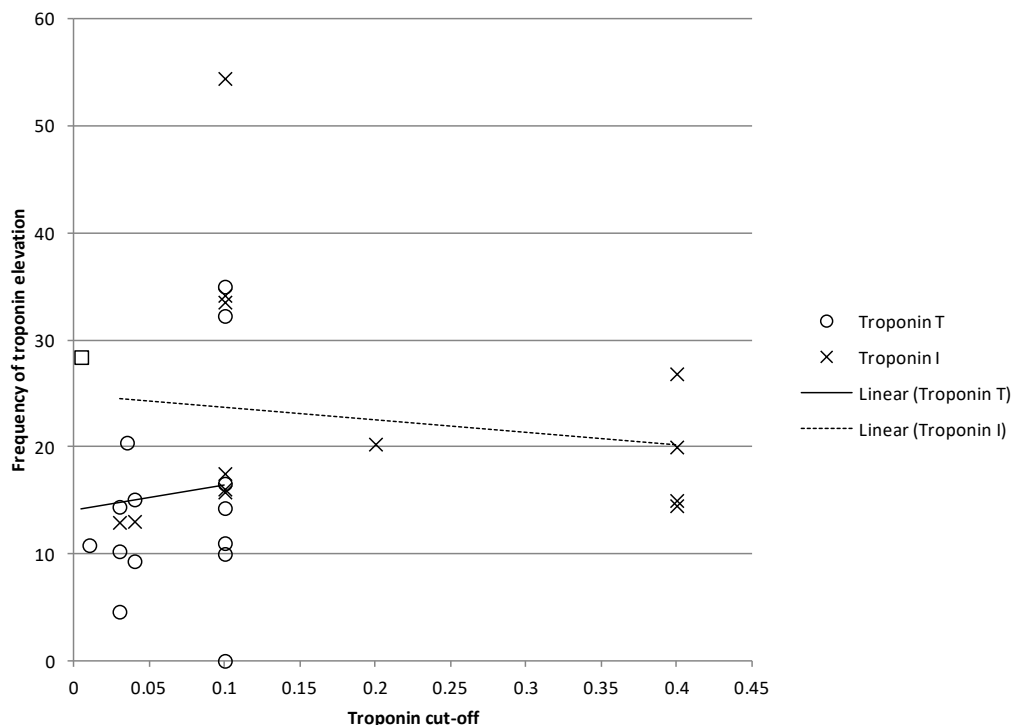


Figure 3. Frequency of troponin elevations determined by year of study.



Square indicates highly sensitive troponin T study. Linear trend lines plotted using least squares method.

Figure 4. Frequency of troponin T and I elevations determined by cut-off used.

The association with stroke severity is similarly complex. Most studies have also shown an association with lesion volume for both ischaemic and haemorrhagic stroke [13, 68]. The association with cardioembolic stroke does suggest that the cause of troponin rise may be related to cardiac pathology. However, cardioembolic strokes are generally more severe [33] and therefore this apparent association could be an artefact. This aetiology has not emerged as an independent risk factor for troponin rise from multivariate analyses, but not all studies incorporated aetiology in the model and sample sizes were generally small.

Involvement of the insular region is an intriguing finding which has been commented on previously particularly in the context of sympathomimetic cardiac stress as a cause of troponin rise in stroke [187].

This will be discussed in more detail below but suffice to say that there is also a relationship between involvement of the insular and large middle cerebral artery stroke [60]. Thus this finding may also be an artefact. There is also a high likelihood of confounding between atrial fibrillation and cardioembolic aetiology, as these two factors are clearly related. The format of the data utilised in this analysis do not permit exploration of interactions through multivariate analysis.

Table 3. Associations of clinical features with elevated troponin at presentation

Clinical Feature	All			Normal Troponin			Elevated Troponin			OR	95% CI	t	P-value
	n			n			n						
N		5559			4620			939					
Demographic Data													
Age (Years) - mean (SD)	3729	70.8	(11.5)	2556	70.9	(10)	371	75.7	(8.3)			8.8	<0.0001
Gender (male) - n/N (%)		2529/5281	(48)		1173/2143	(55)		182/369	(49)	0.80	(0.65 - 1.00)		0.09
Prior History/Assessment													
Myocardial Infarction - n/N (%)		54/1140	(5)		39/909	(4)		15/231	(6)	1.55	(0.83 - 2.86)		0.43
Myocardial Ischaemia - n/N (%)		320/1651	(19)		235/1382	(17)		85/269	(32)	2.25	(1.68 - 3.02)		<0.0001
Atrial Fibrillation - n/N (%)		568/2603	(22)		447/2224	(20)		121/379	(32)	1.86	(1.47 - 2.40)		<0.0001
Ischaemic Heart Disease - n/N (%)		489/2060	(24)		396/1734	(23)		93/326	(29)	1.34	(1.03 - 1.76)		0.06
Congestive Heart Failure - n/N (%)		122/913	(13)		85/745	(11)		37/168	(22)	2.19	(1.43 - 3.7)		<0.001
Renal Failure - n/N (%)		210/1254	(17)		138/1008	(14)		72/246	(29)	2.61	(1.88 - 3.62)		<0.0001
Cardioembolic Aetiology - n/N (%)		375/1240	(30)		293/1080	(27)		82/160	(51)	2.82	(2.01 - 3.96)		<0.0001
Stroke Severity													
Severe - n/N (%)		60/158	(38)		48/136	(35)		12/22	(55)	2.20	(0.89 - 5.47)		0.27
NIHSS - mean (SD)	1570	8.4	(6.5)	1357	7.7	(5.8)	213	13.1	(8.3)			11.8	<0.0001
SSS - mean (SD)	466	38.5	(11.8)	396	40.1	(10.8)	70	29.4	(13.2)			7.4	<0.0001
Lesion Location													
Dominant - n/N (%)		582/1032	(56)		466/816	(57)		116/216	(54)	0.87	(0.64 - 1.18)		0.66
Insular Involved - n/N (%)		361/1446	(25)		272/1243	(22)		89/203	(44)	2.79	(2.05 - 3.79)		<0.0001

OR = odds ratio; CI = confidence interval; NIHSS = National Institutes of Health Stroke Scale; SSS = Scandinavian Stroke Scale; SD = standard deviation.

4.2. Troponin and Other Markers of Cardiac Disease in Acute Stroke

4.2.1. Serological Markers

Troponin T has been shown to be less frequently elevated in acute stroke when compared to both creatinine kinase (CK) and CK-MB isoenzymes with clear evidence that troponin levels can remain completely normal despite significant elevations of these enzymes [20]. It has been concluded that elevations in CK and CK-MB may arise in acute stroke as a result of skeletal muscle lysis because of trauma, prolonged prostration or a combination of relative dehydration and starvation. This notion is supported by the finding of concomitant elevations of myoglobin in a pattern which follows the elevations of CK/CK-MB over about a five day period [20]. This contrasts with the pattern of troponin elevation typically seen in stroke which is generally a tonic elevation lasting for at least the first 5 days [20, 107]. A detailed study of degradation patterns of troponin I has suggested that troponin I elevations in acute stroke have the same origin as those seen in myocardial infarction, indicating that the troponins seen are indeed of cardiac origin [106]. Correlations between troponin levels and both CK and lactate dehydrogenase levels have been demonstrated [13].

Elevations in NT-proBNP have also been described in the setting of acute stroke [58, 81, 98]. This neurohormone is released from ventricular myocytes in response to ventricular pressure and volume overload. NT-proBNP acts via the rennin-angiotensin-aldosterone system, endothelin and sympathetic outflows to cause vasodilatation as well as fluid and water excretion in the kidneys [71]. In the setting of acute stroke NT-proBNP elevations are more common than troponin leak [58] and appear to predate the peak of troponin and CK elevations by approximately 48 hours [98]. In general use, NT-proBNP is a valuable marker for cardiac failure [134]. In one study the levels of NT-proBNP were statistically significantly associated with serum troponin levels ($r = 0.29$) and inversely associated with LVEF ($r = -0.67$) [98] suggesting that left ventricular failure may be part of the explanation for troponin elevations in acute stroke. However, the correlation coefficient between NT-proBNP and troponin was low (0.29) and elevations of NT-proBNP in acute stroke are significantly more common (65%) [58] compared with troponin (11%) in the meta-analysis presented in this chapter. One study has suggested that NT-proBNP is an independent predictor of outcome even when troponin and stroke severity (NIHSS) were included in the model [81], but two larger studies failed to demonstrate any independent predictive value of NT-proBNP with regards to outcome [58, 98]. Cardiac failure is an independent predictive factor following stroke [144] and this was also identified in one of the studies of NT-proBNP [58].

4.2.1. ECG Findings

A summary of specific ECG findings from the studies evaluated is provided in Table 4. This confirms the previously noted association between atrial fibrillation and elevated troponin.

What is also highlighted is the fact that there seems to be an additional association with new onset atrial fibrillation (OR 5.4; 95% CI; 1.85 – 15.82). It is noteworthy that in the data from studies looking only at haemorrhagic stroke [136] the association with atrial fibrillation was not apparent, although the numbers were small. The finding of an association between troponin rise in stroke, particularly ischaemic stroke, and atrial fibrillation adds to the notion of a connection between troponin rise and a cardioembolic cause of the stroke. The fact that atrial fibrillation is not a factor in troponin rise associated with haemorrhagic stroke suggests

that the mechanism in ischaemic stroke is more likely to be through the association of cardioembolic stroke with larger infarcts and greater stroke severity.

Table 4. ECG associations with elevated serum troponin in acute stroke

ECG Feature	Normal Troponin		Elevated Troponin		OR	95%CI	P-value
	n/N	(%)	n/N	(%)			
Atrial fibrillation	103/782	(13)	44/147	(30)	2.82	(1.87 - 4.24)	<0.0001
New onset AF	12/250	(5)	6/28	(21)	5.41	(1.85 - 15.82)	0.003
Old infarct	52/369	(14)	14/88	(16)	0.97	(0.51 - 1.83)	0.947
Current infarction	13/438	(3)	9/78	(12)	4.26	(1.76 - 10.35)	0.002
Ischaemic changes	211/1027	(21)	75/206	(36)	2.21	(1.61 - 3.05)	<0.0001
Prolonged QTc	5/39	(13)	0/10	(0)	0.3	(0.02 - 5.86)	0.756
RBBB	35/301	(12)	10/59	(17)	1.55	(0.72 - 3.34)	0.36
LBBB	13/439	(3)	7/93	(8)	2.67	(1.03 - 6.88)	0.071

AF = atrial fibrillation; RBBB = right bundle branch block; LBBB = left bundle branch block.

There is again an association between troponin elevation and both current acute myocardial infarction and ischaemic ECG changes. However, the frequency of myocardial infarction as a cause of troponin rise is low (12%) and is clearly not the cause of troponin leak in the majority of acute strokes. The situation is further complicated by the finding that ST elevation in the setting of subarachnoid haemorrhage was associated with normal coronary vessels at angiography [121] indicating that the frequency of myocardial infarction may be overestimated in these settings. Evidence of old infarction, prolongation of the QT interval and bundle branch block (right and left) were not associated with elevated troponin levels. Prolongation of the QT interval has been previously shown to be an independent predictor of death following stroke [89] and in a multivariate analysis both prolonged QT and elevated troponin were independent predictors of death or dependence, as measured by the modified Rankin scale [65].

4.2.1. Echocardiography

Four studies have specifically looked at the results of echocardiography in relation to troponin elevations in acute stroke [13, 34, 45, 98]. A significant association between troponin elevations and wall motion abnormality on echocardiography has been described in one recent study [45]. An inverse correlation between LVEF and troponin level has been found ($r = -0.53$; $p < 0.0001$ [13]. Combining two studies which looked at LVEF in acute stroke the mean for patients with elevated troponin was significantly lower ($52.1\% \pm 8.7\%$) than in those with normal troponin levels ($61.0\% \pm 4.8\%$; $p < 0.0001$) [13, 98]. Similarly, using a cut-off of LVEF $< 50\%$ troponin elevation was seen in 11/29 (18%) patients with reduced LVEF versus 22/122 patients with normal LVEF ($p = 0.037$) [13, 34]. No association with left atrial enlargement, left ventricular hypertrophy or focal hypokinesis was seen [34].

4.3. Troponin and Lesion Characteristics in Acute Stroke

Detailed analysis of lesion characteristics on imaging has been undertaken in a number of studies of troponin elevation in acute stroke [13, 34, 67, 68, 98, 136, 187]. A summary of the findings from these studies is given in Table 5.

Table 5. Lesion locations of strokes with and without troponin elevation

Lesion Location	Normal Troponin		Elevated Troponin		OR	95% CI	χ^2	P-value
	n/N	(%)	n/N	(%)				
Cortical								
Frontal/parietal/temporal (MCA/ACA)	176/507	(35)	32/67	(48)	1.719	(1.029 - 2.872)	3.814	0.051
Occipital (PCA)	24/507	(5)	5/67	(7)	1.623	(0.598 - 4.408)	0.438	0.508
Insular	83/468	(18)	20/57	(35)	2.507	(1.385 - 4.539)	8.633	0.003
Basal ganglia/internal capsule (lacuna)	183/507	(36)	14/67	(21)	0.468	(0.253 - 0.866)	5.409	0.02
Posterior fossa (brainstem/cerebellum)	114/468	(24)	15/57	(26)	1.109	(0.593 - 2.074)	0.026	0.872
Dominant	111/238	(47)	19/36	(53)	1.279	(0.634 - 2.581)	0.259	0.611

MCA = middle cerebral artery; ACA = anterior cerebral artery; PCA = posterior cerebral artery; OR = odds ratio; CI = confidence interval.

Troponin elevation is associated with lesions in the frontal, parietal and temporal cortex (middle cerebral artery and anterior cerebral artery territories for ischaemic strokes) and specifically with the insular region as noted previously.

Conversely there was a lower risk of troponin elevation for lesions of the basal ganglia and internal capsule (lacuna infarcts). There was no association with occipital or posterior fossa infarcts (both posterior cerebral artery territory) and there was no clear pattern in terms of laterality of stroke.

Again posterior circulation strokes tend to be small in volume and less dramatic in terms of their clinical manifestations. Similar trends for lesion location associations were seen for the one study of haemorrhagic stroke [136] as compared to the results for ischaemic stroke.

Greater lesion volume also shows an association with elevated troponin levels with the mean ($\pm$ SD) volume for cases with elevated troponin being 75 ml ($\pm$ 56 ml) and for those with normal troponin levels being 35.7 ml ($\pm$ 72.1 ml; $p = 0.0044$).

4.4. Troponin As an Outcome Predictor in Acute Stroke

In univariate analyses of predictors of outcome following acute ischaemic stroke, elevations of serum troponin T have consistently emerged as being positively associated. A meta-analysis of available data using both death or death and dependence as the outcomes of interest demonstrate an odds ratio for troponin elevation of around 3.5 and 2.5 respectively (Figures 5 and 6).

Funnel plots for these data, whilst widely spread for the death outcome data, show no evidence for bias in either case (Figure 7). A typical survival plot for elevated troponin against normal levels from our own data is shown in Figure 8.

In multivariate analyses troponin elevation has emerged as an independent predictive factor for outcome in 12 out of 16 studies [18, 22, 41, 48, 51, 58, 65, 67, 68, 81, 86, 89, 101, 107, 178, 213]. However, in 3 of these studies no measure of stroke severity or lesion volume was included in the model [18, 65] and in 3 others only crude measures of severity such as GCS and lesion volume were included [67, 86, 101].

As demonstrated earlier, the association of such crude measures of stroke severity with troponin elevation is not significant and thus may not accurately reflect the potential confounding effect of stroke severity. In studies where the NIHSS or SSS were utilised in the multivariate analysis, troponin emerged as an independent predictor in 7 out of 11 studies, this was generally seen in the larger studies.

The NIHSS and SSS are generally found to be independently predictive even in the smaller studies [41, 68, 81]. It is evident that troponin levels are not as helpful as stroke severity measures. A meta-analysis of pooled adjusted OR for death as the outcome gives 2.90 (2.13 – 3.95) and for death or dependence 2.30 (0.90 – 5.85) as shown in Figure 9. For death as the outcome, when studies without a clinical measure of stroke severity are removed the OR falls to 2.44, but the confidence intervals still exceed 1.0 (1.72 – 3.46).

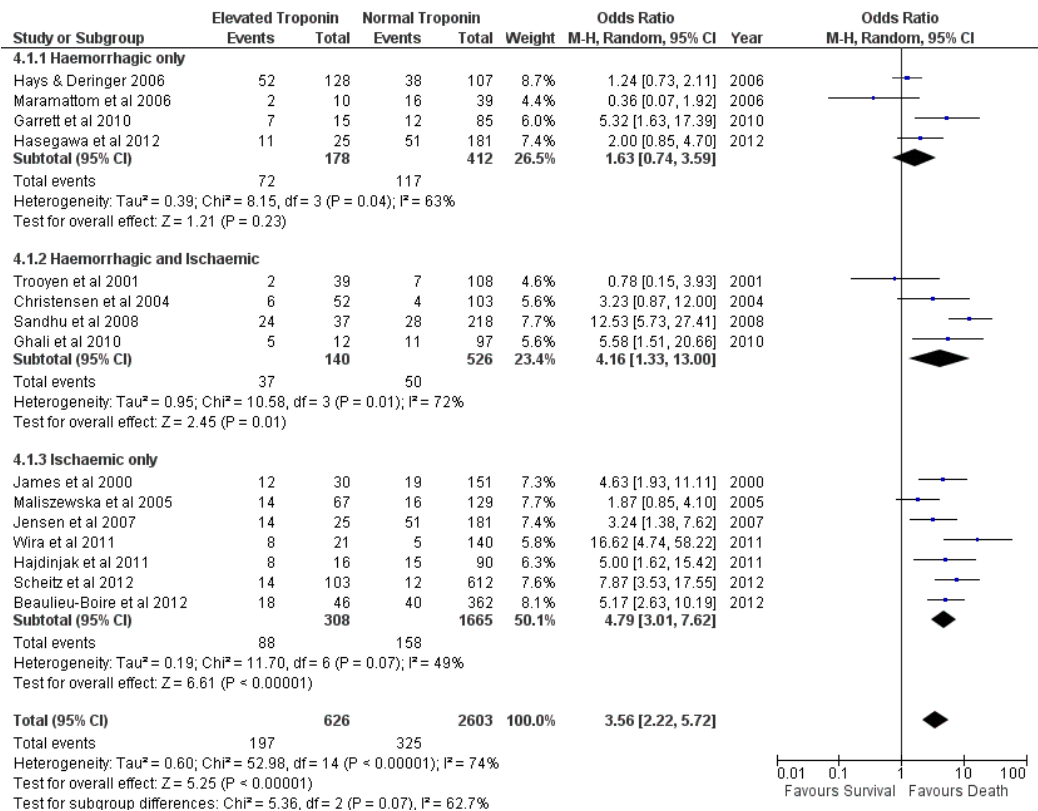


Figure 5. Meta-analysis of troponin in predicting death.

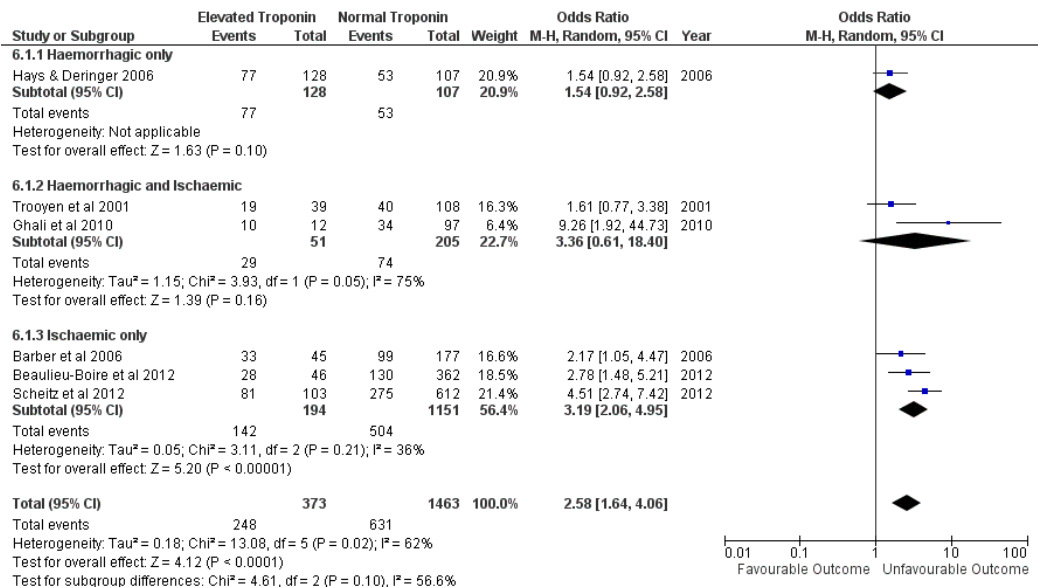
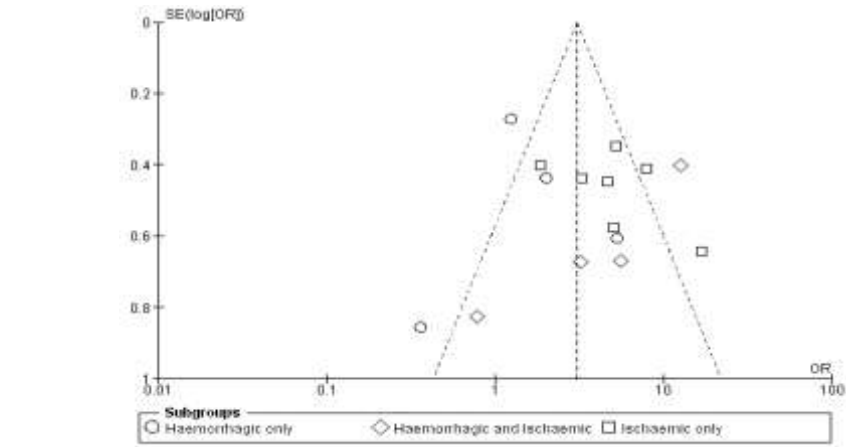
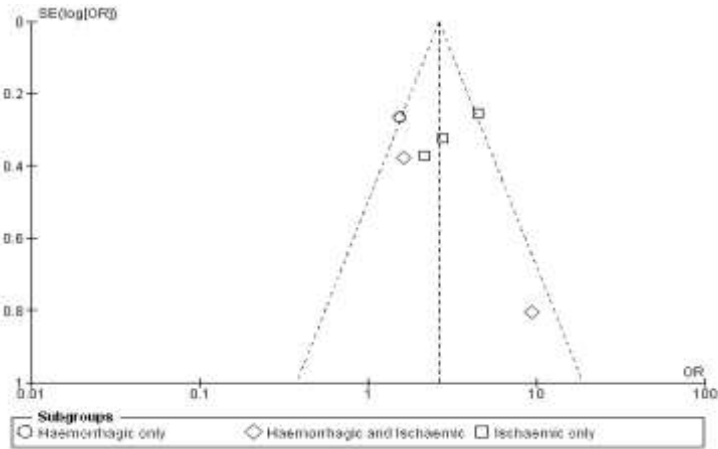


Figure 6. Meta-analysis of troponin in predicting death or dependence.



a



b

OR = odds ratio; SE = standard error

Figure 7. Funnel plots of troponin predicting (A) death and (B) death or dependence.

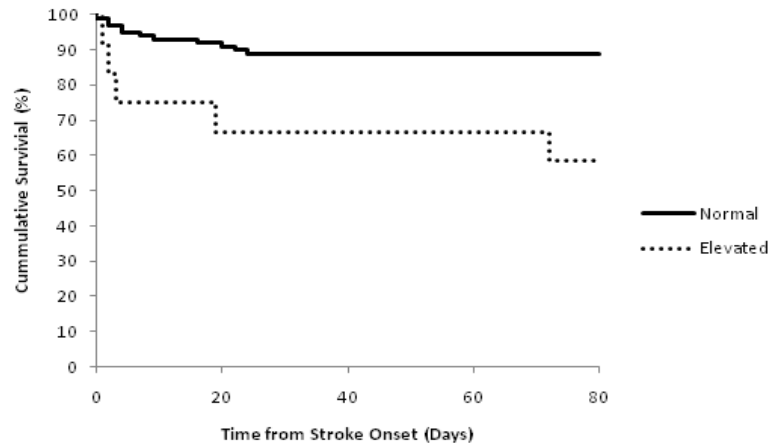
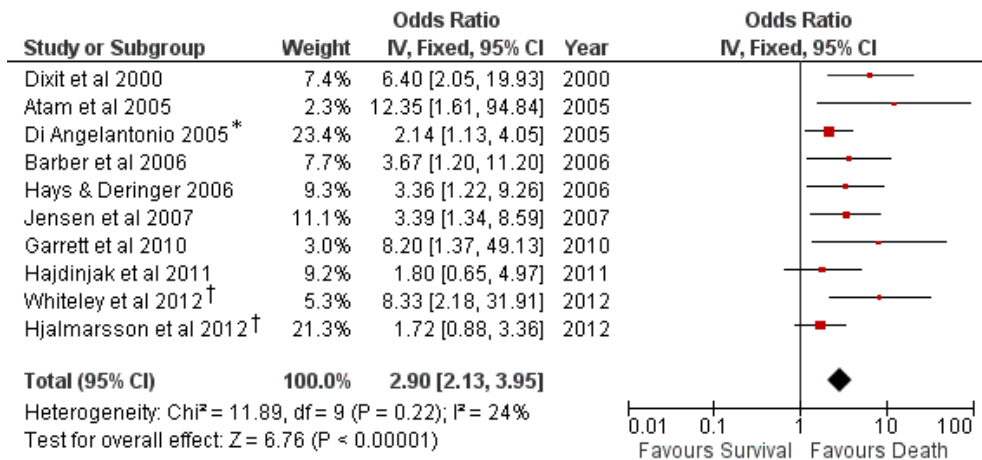
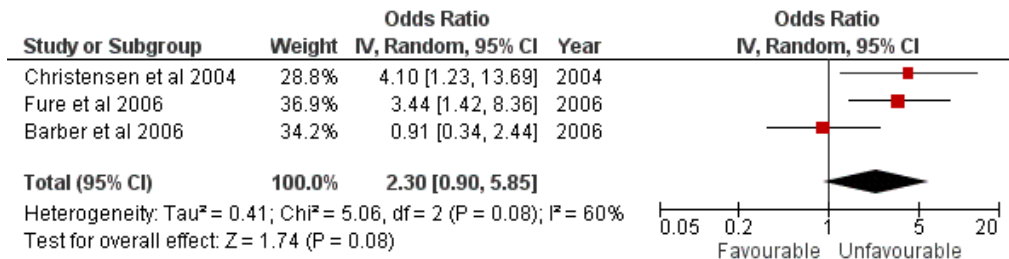


Figure 8. Kaplan-Meier plot of post-stroke survival with normal and elevated troponin levels.



a

* hazard ratio rather than odds ratio † studies excluded from main meta-analysis because of lack of raw data.



b

Figure 9. Forest plots of troponin elevation predicting (A) death and (B) death or dependence outcomes in multivariate analyses.

Analysis of the value of these factors in predicting outcome in terms of sensitivity and specificity are summarised in Table 6. The overall sensitivity of elevated troponin in predicting death was low at 39.4% (95% CI; 35.1 – 43.8%) and this finding was consistent across all types of stroke. However, the specificity was reasonably high at 84.2% (95% CI; 82.7 – 85.5%). The positive likelihood ratio is relatively low at 2.49 but the 95% CI do not cross 1 (2.16 – 2.86) indicating that testing troponin levels in stroke is helpful in predicting death. The negative predictive value is also helpful at 88.4% (95% CI; 87.0 – 89.6%). The figures for death or dependency were similar but generally less impressive with sensitivity of 28.2% and specificity of 86.9% with wider confidence intervals owing to the smaller number of studies which included this outcome measure. It is noteworthy that the comparable figures for an NIHSS score greater than 14 in the prediction of death as an outcome for acute stroke are a sensitivity of 78% (95% CI 58 – 90%) and specificity of 96% (95% CI; 88 – 99%) with a positive likelihood ratio of 17.1 (95% CI, 5.8 – 54.8). Assuming an overall mortality figure of 15% for acute stroke, which is the figure seen for the studies included in this chapter, then the post-test probability for elevated troponin is approximately 25%, whereas for an NIHSS score greater than 14 on admission the post-test probability of death is approximately 70%.

Table 6. Troponin as a predictor of death in acute stroke

[Ref]	Year	Elevated Troponin		Normal Troponin		Sens	(95% CI)	Spec	(95% CI)	PPV	(95% CI)	NPV	(95% CI)	LR+	(95% CI)
		n/N	(%)	n/N	(%)										
Haemorrhagic only															
[86]	2006	52/128	(41)	38/107	(36)	0.58	(0.48 - 0.68)	0.48	(0.40 - 0.56)	0.41	(0.33 - 0.49)	0.65	(0.55 - 0.73)	1.10	(0.871 - 1.39)
[136]	2006	2/10	(20)	16/39	(41)	0.11	(0.03 - 0.33)	0.74	(0.57 - 0.86)	0.20	(0.06 - 0.51)	0.59	(0.43 - 0.73)	0.43	(0.10 - 1.81)
[67]	2010	7/15	(47)	12/85	(14)	0.37	(0.19 - 0.59)	0.90	(0.82 - 0.95)	0.47	(0.25 - 0.70)	0.86	(0.77 - 0.92)	3.73	(1.54 - 9.02)
[85]	2012	11/25	(44)	51/181	(28)	0.18	(0.10 - 0.29)	0.90	(0.84 - 0.94)	0.44	(0.27 - 0.63)	0.72	(0.65 - 0.78)	1.83	(0.88 - 3.79)
Combined		72/178	(40)	117/412	(28)	0.38	(0.32 - 0.45)	0.74	(0.69 - 0.78)	0.40	(0.34 - 0.48)	0.72	(0.67 - 0.76)	1.44	(1.13 - 1.84)
Haemorrhagic and Ischaemic															
[203]	2001	2/39	(5)	7/108	(6)	0.22	(0.06 - 0.55)	0.73	(0.65 - 0.80)	0.05	(0.01 - 0.17)	0.94	(0.87 - 0.97)	0.83	(0.24 - 2.90)
[41]	2004	6/52	(12)	4/103	(4)	0.60	(0.31 - 0.83)	0.68	(0.60 - 0.75)	0.12	(0.05 - 0.23)	0.96	(0.90 - 0.99)	1.89	(1.08 - 3.31)
[173]	2008	24/37	(65)	28/218	(13)	0.46	(0.33 - 0.60)	0.94	(0.89 - 0.96)	0.65	(0.49 - 0.782)	0.87	(0.82 - 0.91)	7.21	(3.95 - 13.16)
[68]	2010	5/12	(42)	11/97	(11)	0.31	(0.14 - 0.56)	0.93	(0.85 - 0.96)	0.42	(0.19 - 0.68)	0.89	(0.81 - 0.94)	4.15	(1.50 - 11.49)
Combined		37/140	(26)	50/526	(10)	0.43	(0.33 - 0.53)	0.82	(0.79 - 0.85)	0.26	(0.20 - 0.34)	0.91	(0.88 - 0.93)	2.39	(1.77 - 3.23)
Ischaemic only															
[101]	2000	12/30	(40)	19/151	(13)	0.39	(0.24 - 0.56)	0.88	(0.82 - 0.92)	0.40	(0.25 - 0.58)	0.87	(0.81 - 0.92)	3.23	(1.74 - 6.00)
[135]	2005	14/67	(21)	16/129	(12)	0.47	(0.30 - 0.64)	0.68	(0.61 - 0.75)	0.21	(0.13 - 0.32)	0.88	(0.81 - 0.92)	1.46	(0.94 - 2.28)
[107]	2007	14/25	(56)	22/219	(10)	0.39	(0.25 - 0.55)	0.95	(0.91 - 0.97)	0.56	(0.37 - 0.73)	0.90	(0.85 - 0.93)	7.35	(3.63 - 14.90)
[215]	2011	8/21	(38)	5/140	(4)	0.62	(0.36 - 0.82)	0.91	(0.86 - 0.95)	0.38	(0.21 - 0.59)	0.96	(0.92 - 0.99)	7.01	(3.57 - 13.75)
[24]	2012	18/46	(39)	40/362	(11)	0.31	(0.21 - 0.44)	0.92	(0.89 - 0.94)	0.39	(0.26 - 0.54)	0.89	(0.85 - 0.92)	3.88	(2.30 - 6.54)
[81]	2012	8/16	(50)	15/90	(17)	0.35	(0.19 - 0.55)	0.90	(0.82 - 0.95)	0.50	(0.28 - 0.72)	0.83	(0.74 - 0.90)	3.61	(1.52 - 8.57)
[178]	2012	14/103	(14)	12/612	(2)	0.54	(0.34 - 0.71)	0.87	(0.84 - 0.89)	0.14	(0.08 - 0.22)	0.98	(0.97 - 0.99)	4.17	(2.78 - 6.25)
Combined		88/308	(29)	129/1703	(8)	0.41	(0.34 - 0.47)	0.88	(0.86 - 0.89)	0.29	(0.24 - 0.34)	0.92	(0.91 - 0.94)	3.31	(2.70 - 4.05)
Total		197/626	(31)	296/2641	(11)	0.40	(0.36 - 0.44)	0.85	(0.83 - 0.86)	0.36	(0.28 - 0.35)	0.888	(0.88 - 0.90)	2.58	(2.25 - 2.97)

CI = confidence interval; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; LR+ = positive likelihood ratio.1.

One recent study using a highly sensitive troponin T assay has suggested that this test may be of more value in predicting outcome when compared to a fourth generation troponin T assay [66]. However, another study using the same assay in stroke patients who were negative for a fourth generation troponin T assay showed no additional predictive value for the more sensitive assay despite identifying troponin elevations in 65/193 (33.7%) of apparently troponin negative patients on the standard assay [108]. A recent study of a wide range of biomarkers in a large cohort of subjects showed no additional predictive value over and above clinical measures for any of the assays, which included troponin T [213].

The commonest causes of death following stroke in the acute phase are complications of the stroke and recurrent stroke (approximately 85%), but after one year cardiovascular disease is the commonest (approximately 55%) [83]. In one study of troponin elevations in acute stroke cause of death was analysed and although the numbers were small 4/9 (44%) of deaths in the group with elevated troponins were cardiac deaths compared with 1/4 (25%) of patients without troponin elevations. This result was not statistically significant but the numbers of cardiac deaths in the normal troponin group is similar to that expected from larger studies of stroke survival in the acute phase. This suggests that troponin elevations are related to cardiac injury and an increased risk of cardiac death as a consequence.

5. MECHANISMS OF TROPONIN RISE IN ACUTE STROKE

5.1. Myocardial Infarction

From the data presented in this chapter it is clear that troponin rise in acute stroke is due to myocardial infarction in a minority of cases (around 12%). In the largest single study of troponin levels in stroke the estimated frequency of myocardial infarction within the preceding 2 weeks for those with an elevated level was 8/73 (11%) [21]. These figures are consistent with a previous estimate [104]. The fact that the difference in the frequency of troponin elevation in acute stroke was only marginally different when studies that excluded myocardial infarction and other known causes of troponin elevation were removed also suggests this is a relatively uncommon cause. Myocardial infarction is a well-recognised cause of stroke [208]. A recent meta-analysis has shown that the in-hospital risk of stroke following acute myocardial infarction is 14.5% [216]. The purported mechanisms include emboli from arrhythmias (most notably atrial fibrillation) as well as intraventricular thrombus associated with ependymal infarction [207], altered flow dynamics due to focal hypokinesia [131] or a ventricular aneurysm [133]. A prothrombotic state may also lead to the co-occurrence of both pathologies [198]. Myocardial infarction may also precipitate cardiac failure and is associated with a number of physiological responses which can lead to a prothrombotic tendency which might contribute to the heightened risk of stroke [30, 202]. It is pertinent to note that haemorrhagic stroke is less common than ischaemic stroke as a complication of myocardial infarction, yet troponin elevation is more commonly seen in haemorrhagic stroke compared with ischaemic events. It therefore seems likely that it is the severity of the cerebral insult that is important in generating a troponin rise. This possibility has been noted previously [118]. It has been postulated that very high troponin elevations are generally indicative of acute myocardial infarction in the acute stroke setting, but a precise

cut-off has not been determined. Early revascularisation in the setting of acute coronary syndrome has been shown to reduce the risk of subsequent stroke independently of the size of infarct or the presence of cardiac failure [209].

5.2. Renal Failure

Renal failure may be a predisposing factor for troponin elevation in up to 30% of acute strokes, but severe renal failure as a sole cause of troponin elevation is rare in stroke [21, 68]. Because of this, it is not possible to comment on the causal mechanism. Renal failure may simply predispose to an increased risk of stroke through hypertension and other mechanisms whilst also causing a background elevation in troponin levels as presented earlier in this chapter. The possibility of an increased risk of cardioembolic stroke due to cardiac failure cannot be excluded as a possible mechanism for the association.

5.3. Infective Endocarditis

Bacterial and other forms of endocarditis are occasional causes of acute stroke [84, 159]. Troponin elevations in stroke have been noted to be associated with elevations of C-reactive protein levels which can be a sign of infection, underlying arteritis and ischaemic inflammation [203]. However, it seems unlikely that bacterial endocarditis and associated cardiac failure or sepsis are responsible for troponin rise in anything other than occasional cases. One retrospective study identified 27 cases of definite infective endocarditis (modified Duke criteria [127]) as a cause of stroke over 7 years in one centre [56]. The majority of cases of endocarditis were diagnosed after the onset of stroke symptoms. No denominator was provided but these figures would suggest a co-occurrence of endocarditis in no more than a few percent of cases of stroke.

5.4. Pulmonary Embolism

Pulmonary embolism was identified as a possible cause of troponin rise in acute stroke in 1/73 cases in one series [21]. Again this must be only an occasional cause of troponin rise in acute stroke.

5.5. Serious Arrhythmias

Serious tachyarrhythmias are seen in only a small proportion of patients with acute stroke (around 1%) [215]. It therefore seems unlikely that this is a common cause of troponin elevation in acute stroke. Whilst a number of conduction abnormalities and atrial fibrillation have been identified amongst patients with elevated troponin levels in acute stroke, tachyarrhythmias have generally not been observed [48, 65, 89, 203].

5.6. Cardiac Failure

A background of ischaemic heart disease and congestive cardiac failure is clearly important and it seems likely that current cardiac failure is the cause of troponin rise in perhaps 40% or so of cases. There is supporting evidence for this from a study indicating an association between troponin elevations in acute stroke and lower haemoglobin level, haematocrit and white blood cell count [48], which could be explained by the haemodilution effect of cardiac failure [11]. Cardiac failure is also a well-recognised cause of stroke with a cumulative incidence of around 16% after a little over 4 years [217]. The suggested mechanisms are again ventricular thrombus formation and hypercoagulability.

Cardiac failure and left ventricular failure associated with pulmonary oedema is well recognised in subarachnoid haemorrhage indicating that this phenomenon can arise as a result of extreme generalised cerebral insult [42]. Cardiac failure is also associated with an increased risk of recurrent stroke due to cardioemboli [102] and it seems likely that relative hypoperfusion due to cardiac failure would be associated with a greater extent of infarction in the ischaemic penumbra of ischaemic stroke. Cardiac failure is certainly associated with an adverse outcome in stroke [183] and also predisposes patients to a number of other cardiac and non-cardiac complications which would contribute to an increased risk of death and a poorer prognosis.

5.7. Other Possible Causes of Troponin Rise in Acute Stroke

After excluding frank myocardial infarction, serious arrhythmias, endocarditis, pulmonary embolism and renal failure the cause of troponin rise remains unexplained in over half of cases.

5.7.1. Atrial Fibrillation

The meta-analysis presented in this chapter indicates that atrial fibrillation is seen in roughly one third of cases of acute stroke with elevated troponin and also suggests that the association with new onset atrial fibrillation is higher. A previous study looking specifically at atrial fibrillation found an association between troponin elevation and new onset atrial fibrillation [28]. An association with atrial fibrillation, particularly recent onset atrial fibrillation, and cardioembolic stroke raises a number of intriguing possibilities. Uncomplicated atrial fibrillation is not generally associated with troponin leak. However, in the setting of rapid atrial fibrillation [113, 154] or cardiac failure precipitated by atrial fibrillation [170] troponin leak can occur. The association could be purely due to troponin elevation being linked to lesion size and stroke severity, as it is established that cardioembolic strokes are more severe [33]. One further potential connection is that atrial fibrillation may lead to simultaneous embolisation to the brain and coronary vasculature [119]. One detailed study of seemingly cryptogenic stroke in which cases with atrial fibrillation were excluded found an association between troponin elevation and evidence for a cardioembolic source on detailed echocardiography. This suggests that at least in some cases it may be the cardioembolic nature of the stroke that is the cause of troponin rise rather than the cardiac

consequences of any underlying pathology [174]. An association between new onset atrial fibrillation and lesions involving the insular cortex has also been described [60].

Conversely it is not inconceivable that acute stroke may result in atrial fibrillation. A variety of arrhythmias including atrial fibrillation have been described in acute stroke [123] and subarachnoid haemorrhage [54]. In this scenario the troponin rise and onset of atrial fibrillation might both be triggered by some common pathophysiological mechanism. However, as atrial fibrillation is pre-existent in the majority of cases of acute stroke with troponin rise this is unlikely to be a common mechanism.

Atrial fibrillation is known to contribute to a worse outcome in acute stroke [172] and is associated with an increased risk of cardiac complications [204]. This raises the possibility that the mechanisms for a worsening of outcome in acute stroke for both atrial fibrillation and troponin elevation may be the same or have some overlap. One likely mechanism in common is cardiac failure.

5.7.2. Takotsubo Cardiomyopathy

A reversible form of stress-induced cardiomyopathy has been described to occur in the setting of high emotional or physical distress [177]. This syndrome termed stress-induced cardiomyopathy or takotsubo cardiomyopathy (in reference to the similarity of left ventriculographic appearances to the shape of a Japanese octopus pot) leads to apical ballooning of the left ventricle in response to sympathetic overflow to the heart (Figure 10). The syndrome is more common in postmenopausal women and has an in-patient mortality of around 4% [27, 182]. If the acute phase is survived, the pathological changes appear to resolve completely with little or no long term effects. Pathological examination of cases of sudden death in similar stressful circumstances has demonstrated a particular sub-endocardial lesion, contraction-band necrosis (also known as myofibrillar degeneration) [17, 32]. A very similar pathology can be induced in animals with high levels of catecholamines and there is evidence to suggest that the trigger for these microscopic pathological changes is a massive influx of calcium into the myocytes triggered by reperfusion [226]. Similar findings have been described in animal models of subarachnoid haemorrhage [74]. However, this pathology is not seen in all cases of takotsubo cardiomyopathy and milder changes can be seen [220]. A key component of the diagnosis is the demonstration of normal coronary arteries [163].

A non-ischaemic multifocal transmural myocardial pattern of damage has been described in 13/25 (52%) of fatal cases with cerebral infarction and 9/13 (69%) with intracerebral haemorrhage [120]. Takotsubo cardiomyopathy has been described in association with both ischaemic and haemorrhagic stroke in single case reports [31, 114, 179, 184] and small series [73, 224]. In one series 6/7 patients had lesions involving the insular cortex [224]. Takotsubo cardiomyopathy is known to be associated with elevations in serum troponin [63], but this is not always seen. Generally, levels are somewhat intermediate between those seen in extensive myocardial infarction and normal levels. Takotsubo cardiomyopathy is also associated with elevations of NT-proBNP [153] and the levels are higher than in acute coronary syndromes [5]. Peak troponin levels are achieved on day 1 and NT-proBNP levels are seen to rise quite dramatically at day 2 [63].

The commonest ECG changes seen in takotsubo cardiomyopathy include T wave inversion, QTc prolongation, ST-segment elevation and Q waves [195]. This mirrors closely the pattern of ECG changes typically seen in both ischaemic [65] and haemorrhagic [85] stroke with troponin elevations.

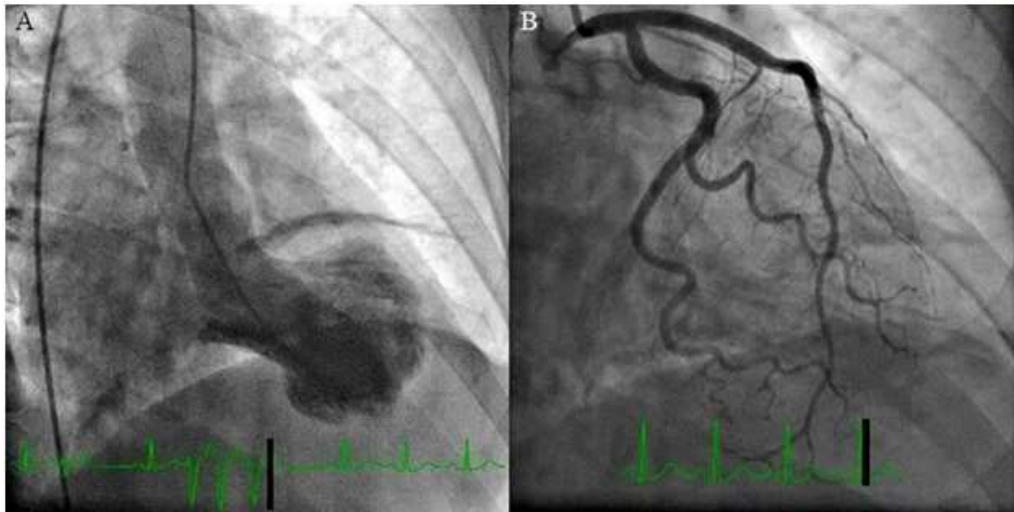


Figure 10. Angiographic images showing (A) apical ballooning of the left ventricle in systole and (B) normal coronary arteries.

Several studies of troponin elevation in acute stroke have included echocardiography [13, 34, 45, 98]. It is noteworthy that none of these studies has specifically identified any cases of takotsubo cardiomyopathy. However, in two studies echocardiography was performed within 3 days of admission [45, 98] and for the other two no time criteria or data were given. It is known that the echocardiographic features of takotsubo cardiomyopathy can resolve within 48 hours [55, 167] and thus the typical appearances might be missed after this window. The ventricular morphology associated with takotsubo cardiomyopathy can be variable [96] with *formes frustes* of takotsubo cardiomyopathy also having been suspected [64].

The most commonly postulated pathogenesis for takotsubo cardiomyopathy is that an intensely stressful emotional or physical trigger causes an excess of circulating catecholamines which causes both direct myocardial toxicity as well as microvascular dysfunction, which leads to myocardial stunning and the consequent contractile dysfunction [219]. The more dense distribution of adrenoceptors at the apex is thought to explain why the apex is usually affected while the base of the ventricle is spared.

Perhaps the most striking argument against takotsubo cardiomyopathy being a significant contributor to troponin elevations in acute stroke is the demographic profile of those affected. Oestrogen is associated with attenuated catecholamine-mediated vasoconstriction [193]. Consequently oestrogen deficiency is thought to explain the higher incidence of takotsubo cardiomyopathy in post-menopausal women. Whilst stroke patients with troponin rise are certainly older, the gender distribution was the same as for those with normal troponin levels. However, the pathophysiology in the setting of stroke may be different and a partial form of takotsubo cardiomyopathy may be responsible.

As well as takotsubo cardiomyopathy arising as a consequence it is also possible that stroke can arise as a complication of this pathology which is associated with a risk of embolisation [139].

5.8. Stroke and the Autonomic Nervous System

In the human body, the most important catecholamines are adrenaline, noradrenaline and dopamine. Catecholamines are produced mainly by the chromaffin cells of the adrenal medulla and the postganglionic fibres of the sympathetic nervous system [129]. Release of these hormones from the adrenal medulla is part of the fight-or-flight response. Following release of the neurotransmitter noradrenaline (adrenaline from the sympathetic adrenergic nerves) it binds to specific adrenergic receptors in the target tissue to produce physiological response.

Vascular smooth muscle has a large number of α_1 receptors relative to β_2 receptors. However, adrenaline has a higher affinity for the β_2 receptors relative to the α_1 receptor [125]. Activation of the β_2 receptor would produce vasodilatation while activation of the α_1 receptor would result in vasoconstriction [4]. At low doses, adrenaline can selectively stimulate β_2 receptors. However, as the concentration of adrenaline increases, the predominant effect is vasoconstriction. Noradrenaline in physiologically relevant concentrations has little affinity for β_2 receptors. It stimulates α_1 receptors producing vasoconstriction.

Normally, small coronary arteries and arterioles are the principal determinants of coronary vascular resistance [39]. Their calibre is determined by the resting tone of the autonomic nervous system [40]. Responses to α -adrenoceptor activation are augmented in the presence of coronary endothelial dysfunction and atherosclerosis, involving both α_1 - and α_2 -adrenoceptors in epicardial conduit arteries and microvessels. Such augmented α -adrenergic coronary constriction is powerful enough to induce myocardial ischemia and limit myocardial function [87]. Both α_1 - and α_2 -adrenoceptors mediate coronary vasoconstriction with α_1 -adrenoceptors more predominant in larger vessels and α_2 -adrenoceptors more predominant in the microcirculation [38]. The overall effect of sympathetic stimulation on the cardiovascular system is to increase heart rate and blood pressure (Figure 11).

The central pathway of cardiac control involves what has been termed the cardunculus in the insular cortex via central connections through the hypothalamus and ultimately outflow tracts through the vagus nerve to the heart [157, 165]. Both sympathetic and parasympathetic drive to the heart may be governed by the insulae via the hypothalamus. An MRI study identified the insular region as being more commonly involved in stroke patients with right cerebral artery territory infarction and elevated troponin level [21]. However, another study failed to find any association between lesions of the insular region and elevations of troponin or ECG changes [41]. This study also provided additional evidence for a significant stress response in acute stroke, in the form of elevations in cortisol and TNF- α levels. In stroke, insular infarction, night-time blood pressure increase and increased serum noradrenaline concentrations are independent risk factors for unfavourable functional outcome (measured by the Barthel index) at one year of follow-up [171], and it has been hypothesised that persistent autonomic activation is related to poor functional outcomes because of the cardiovascular sequelae [171, 200]. An association between acute stroke, elevated noradrenaline levels, CK levels and arrhythmias has been demonstrated [145]. More recently, asymmetric right midbrain activation has been shown during mental and physical stress induced pro-arrhythmic ECG abnormalities [44].

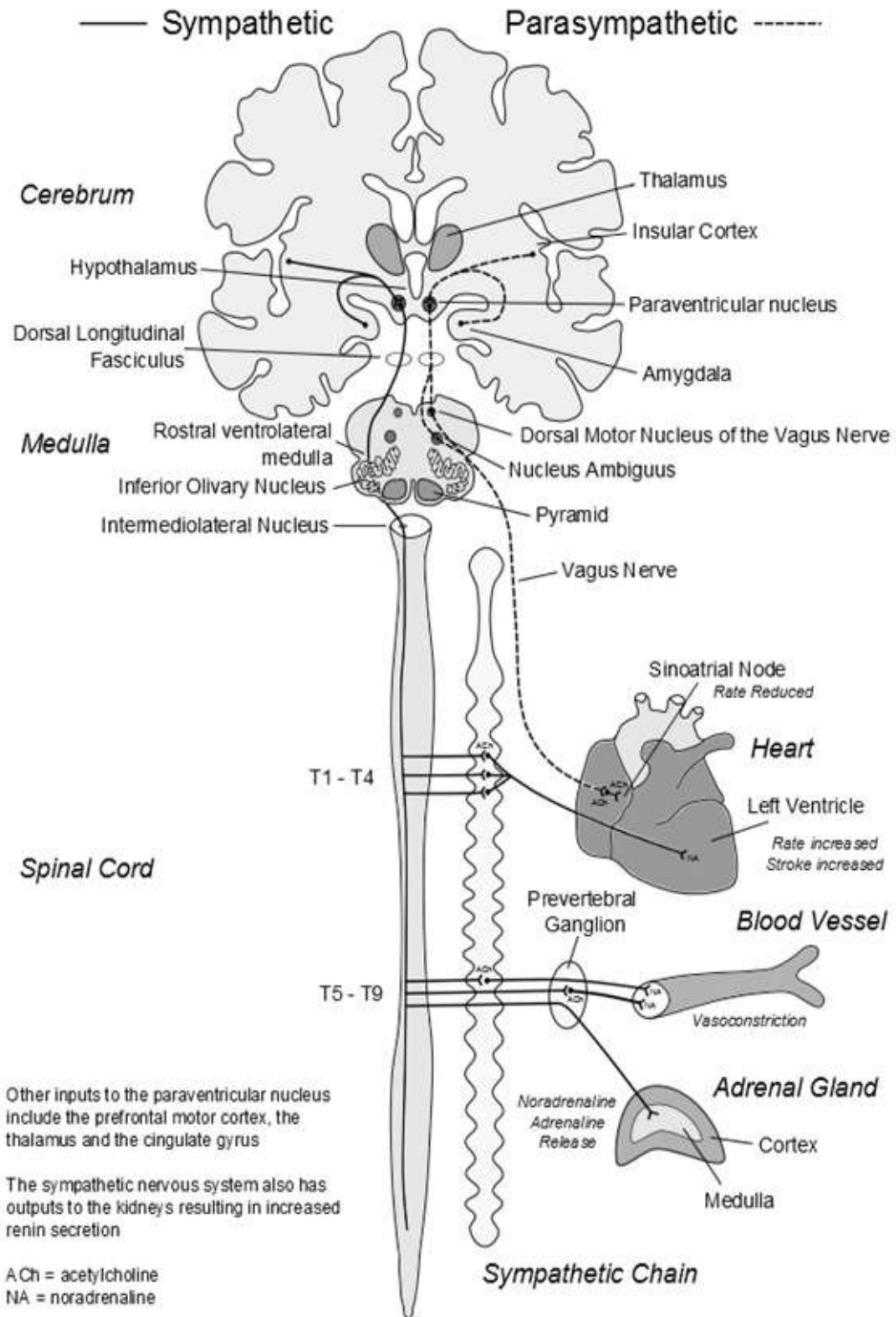


Figure 11. Illustration of the principal cardiovascular efferents of the autonomic nervous system.

An excess of catecholamines and associated myocardial injury have been demonstrated in an animal model of MCA territory infarction [79] and a specific association with lesions of the insular cortex has also been described in an animal model [185]. Elevated catecholamine levels have also been demonstrated in acute stroke patients [146] and elevations do not appear

to correlate well with lesion volume or stroke severity [146, 160]. Various degrees of AV block and tachyarrhythmias have been induced in animals through stimulation of the cingulate gyrus [186], amygdala [166], posterior thalamus [141] and vagus nerve [50].

The importance of the vagus nerve has been demonstrated, particularly for sinus nodal function [165]. Experiments by Oppenheimer demonstrated that the central pathway of cardiac control involves the hypothalamus and the insular cortex [158]. Stimulation of the parasympathetic nervous system has also been demonstrated to induce myocardial ischaemia in animals [75].

It has been noted that the sinoatrial and atrioventricular nodes have predominantly parasympathetic innervation, whereas sympathetic innervation predominates in the ventricular muscle [37]. Stimulation studies in humans undergoing epilepsy surgery have indicated a predominantly sympathetic output from the right insula and parasympathetic output from the left insula [157]. This finding has been supported by studies in psychological paradigms [218] and migraineurs [19]. A specific association between stroke affecting the right insular cortex and an increased risk of heart rate variability and sudden death has been reported [200]. Previous studies have found that patients with right hemispheric infarcts, compared to those with left sided infarcts, have a significant increase in supraventricular arrhythmias [43, 126]. The hypothesis that right hemisphere strokes produce an impairment in the parasympathetic mediated heart rate response with a reciprocal rise in sympathetic tone, making supraventricular tachycardias more likely has been proposed. The data presented in this chapter would not suggest a similar differential lateralisation effect for insular involvement with regards to troponin elevation in acute stroke and the data in the published literature relating to this are complex [156].

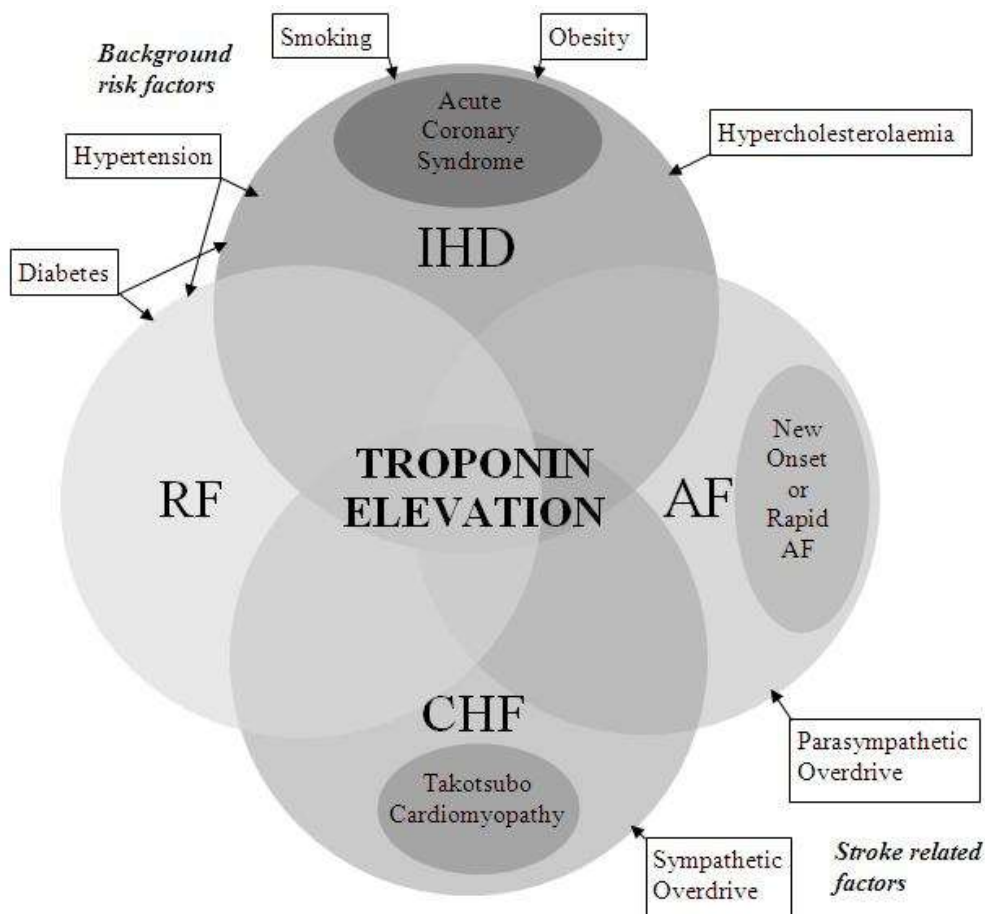
The principal afferents for cardiovascular autonomic control are the baroreceptors of the carotid sinus and the chemoreceptors of the carotid body which pass via the glossopharyngeal nerve, petrosal ganglion and the nucleus of the tractus solitarius to synapse with motor nuclei in the medulla and central control centres, including the hypothalamus [46]. Afferents from the aorta and heart also travel with the vagus nerve. There are additional baroreceptors and chemoreceptors in the area postrema and the hypothalamus which directly influence autonomic outflows [78, 143, 189].

5.9. Mechanistic Summary

A summary of probable mechanisms for troponin leak in acute stroke is given in the form of a Venn diagram with associated risk factors in Figure 12. The available evidence points to pre-existent or co-occurring cardiac conditions accounting for perhaps half of elevations in troponin levels in acute stroke with the remainder arising as sympathetic overflow syndrome characterised by left ventricular dysfunction, ECG changes (which may appear ischaemic) and arrhythmias. In some instances this picture may include frank infarction or a takotsubo cardiomyopathy syndrome but in most cases the cardiac sequelae are less obvious but are still associated with a poorer prognosis. The fact that very similar cardiac features are seen in subarachnoid haemorrhage [176] and raised intracranial pressure [120] points to a more generic stress response rather than a lesion location specific response. In subarachnoid haemorrhage there is also a very clear relationship between the severity of neurological insult and troponin levels [205].

6. TREATMENT IMPLICATIONS

Patients with stroke and troponin elevation should be managed on a case by case basis. In selected cases with chest pain or associated dynamic ECG changes inpatient angiography should be considered. In the majority of patients invasive investigations can be avoided and management of the troponin leak should be conservative. Echocardiography may play an important role in identifying regional or global wall motion abnormalities, intracardiac thrombus, vegetations and aortic dissection. Echocardiography could also be helpful in detecting stress induced cardiomyopathy with apical ballooning following stroke.



IHD = ischaemic heart disease; RF = renal failure; AF = atrial fibrillation; CHF = congestive heart failure.

Figure 12. Venn diagram illustrating aetiology of troponin rise in acute stroke.

The notion of a sympathetic overflow syndrome complicating stroke has led to the concept of using β -blockers or calcium channel antagonists in acute stroke to ameliorate these effects. Animal models of these treatments given prior to the induction of cerebral infarction have proven to be of some benefit [10, 95, 111]. However, trials of β -blockers in humans for unselected patients with acute stroke have shown no benefit and may have had some harmful effect [23]. Similarly studies of calcium channel antagonists have been disappointing in

humans [90, 91]. This may be because of other effects on atrioventricular conduction or blood pressure, or simply because late administration is ineffective. Studies of their value in takotsubo cardiomyopathy have been similarly disappointing [59] and it may well be that once the sympathetic overflow has occurred and contraction band necrosis is present in myocytes that treatment of this sort is ineffective. Pre-admission β -blockers have been found to be effective in preventing stunned myocardium in subarachnoid haemorrhage [128].

Angiotensin converting enzyme inhibitors which are of proven efficacy in cardiac failure [103] are on the other hand also of known benefit in the setting of stroke [76]. The benefit of angiotensin converting enzyme inhibitors is not limited to secondary prevention they also reduce short term morbidity before any significant effects on blood pressure will have been seen [15]. Thus whilst originally introduced for their secondary preventive effects on blood pressure in stroke, it may be their effects on cardiac failure that are also a significant reason for their efficacy in the short term. There is emerging evidence that the acute control of hypertension in the case of intracerebral haemorrhage may be safe and beneficial [9, 12], but further definitive studies are awaited.

Administration of atropine prevented myocardial injury in an animal model of parasympathetic induced myocardial ischaemia [75]. Knowledge regarding the relative contributions of the sympathetic and parasympathetic components of the autonomic nervous system in acute stroke may be required in order to more specifically target any proposed acute treatments.

CONCLUSION

Troponin rise following stroke is seen in a modest but significant proportion of patients with acute stroke. The frequency is around 10% in ischaemic stroke and 18% in haemorrhagic stroke. There is a clear association between troponin rise and the size of cerebral lesion as well as atrial fibrillation and a cardioembolic aetiology. It seems likely that these factors are intertwined, and that it is the greater severity of stroke seen with intracerebral haemorrhage and embolic infarcts that is the principal driver of these associations. Myocardial infarction is the cause of troponin rise in acute stroke in a minority of cases (10%) and the common pathway for troponin elevation in the remainder appears to be cardiac stress or failure: either pre-existent; precipitated by atrial fibrillation; or more commonly a cerebrally induced autonomic disturbance. Whether this is psychologically induced or due to a disturbance of central control remains to be established but the frequency of frank takotsubo cardiomyopathy as a cause appears to be low, but the results of further studies is eagerly awaited.

Troponin elevation is an independent predictor of a poor outcome following stroke both in terms of mortality and morbidity, but its clinical usefulness in this setting has been questioned [212]. The sensitivity at standard cut-off levels for troponin is low but the specificity for the outcome of death is high making this a potentially useful predictive test. Simple clinical measures such as the NIHSS or SSS have similar or better specificity and much higher sensitivity in predicting an adverse outcome and purely clinical tools for predicting outcome in stroke appear to be very adequate [175]. At the present time the influence of finding an elevated troponin in stroke is limited in terms of treatment, but as a marker of cardiac failure and cardioembolic aetiology this test may assist in directing

appropriate investigations. Current stroke management guidelines in Australia [150] and the United Kingdom [149] do not recommend routine troponin measurement in acute stroke but guidelines from the American Stroke Association do [2]. Simply as a screen for acute coronary syndrome is probably justification enough for the routine measurement of troponin levels in acute stroke.

Further research is required to elucidate the nature of the relationship between the identified risk factors for troponin elevation in acute stroke and the mechanisms for this. A formal meta-analysis of raw data from available studies using regression analysis approaches would be a useful starting point for generating hypotheses. A prospective observational cohort study collecting, demographic, vascular risk factor, serological, imaging, ECG, echocardiographic, neurosonological and Holter monitor data in a large group of patients with acute stroke, together with coronary angiography in a subset has been proposed [122]. Another prospective observational study using formal angiography in appropriately selected patients (TRELAS) [178] will certainly be of value in answering some of the remaining questions. Key elements of any such studies would be to include all cases of stroke (haemorrhagic and ischaemic), inclusion of troponin, NT-proBNP, CK and catecholamine levels as part of the serological testing, repeated serological and ECG testing to give a dynamic picture of the myocardial injury [104], measures of stroke severity, lesion volume and assessment of cardiac function with echocardiography or angiography. A defined outcome measure such as modified Rankin scale score at 90 days would also be important. Whilst formal angiographic studies are problematic in the acute stroke setting and may even be contraindicated, improvements in computed tomography [223] and magnetic resonance [116] coronary angiography offer intriguing non-invasive alternatives. The issue of whether or not involvement of the insular cortex in stroke is associated with a greater risk of cardiac sequelae also requires resolution. Detailed lesion analysis could shed light on the central control of cardiac autonomic functioning.

We suspect that cardiologists, neurologists and philosophers alike will continue to argue over whether it is the heart that rules the mind or the mind that rules the heart, but what is clear in the case of troponin elevations in stroke is that, as in real life, the truth is always much more complicated and the two are very much interdependent.

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Chapter 63

HOW COULD FLUOXETINE EXERT THERAPEUTIC EFFECTS IN STROKE?

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ABSTRACT

Stroke is among the leading causes of morbidity and mortality worldwide. The current pharmacological treatment of stroke is early reperfusion and fibrinolysis of the ischemic brain area using the recombinant tissue plasminogen activator (rt-PA, alteplase). Much effort has been invested into the discovery of other pharmacological therapies to provide neuroprotection and to reduce the late neurological and psychiatric deficits associated with stroke. Among the numerous strategies proposed, there is the administration of antidepressant drugs. The recent report that the selective serotonin reuptake inhibitor (SSRI) fluoxetine provides neuroprotection and can improve motor deficits in patients is particularly encouraging. In addition to its well-known therapeutic effect in depression, a frequent long-term consequence of stroke, fluoxetine might exert additional effects after cerebral ischemia independently of its antidepressant effect *per se*. The present review examines various short and long-term mechanisms through which fluoxetine and other SSRIs might exert their therapeutic effects in stroke. In addition to the facilitation of serotonergic transmission, *via* long term regulations of 5-HT receptor functions and neuroplasticity, SSRIs could have important beneficial effects on haemostasis, vasomotricity, trophic and inflammation factors (BDNF, cytokines, microglia, ...) that can reduce the initial consequences (infarct size, sensorimotor deficits) as well as the long term emotional deficits of stroke.

Keywords: Antidepressant drugs; Blood brain barrier; Cerebral ischemia; Cerebral blood flow; Haemostasis; Inflammation; Neurogenesis; Neuroprotection; Neurovascular unit; Post-stroke depression; Trophic factors

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1. INTRODUCTION

Fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) are first line treatments for depression and anxiety. Furthermore, SSRIs are also effective treatments for the long-term emotional deficits associated with stroke, namely post-stroke depression, and could possibly be effective also for the long term cognitive deficits associated with this disease since, as in depression, these might be associated with chronically low extracellular 5-HT levels (Cowen & Sherwood, 2013; Jorge et al., 2010). However, prior to cognitive and depressive symptoms, stroke is mostly recognized by sensory-motor deficits. Cholet et al. FLAME multicenter study about the beneficial effect of fluoxetine for stroke recovery, in particular for motor functions, had recently a considerable impact (Chollet et al., 2011; Cramer, 2011). Indeed, patients that had suffered stroke of moderate severity, but with severe arm and leg motor weakness, treated few days after with fluoxetine experienced great neurological benefits independently of the therapeutic effect of fluoxetine on depressive symptoms. As indicated by functional magnetic imaging, these effects on motor performances are reflected by activity changes at the level of the motor cortex (Pariente et al., 2001). Since then, systematic reviews and meta-analyses have shown the efficacy of SSRIs in reducing dependency and disabilities associated with stroke (Mead et al., 2012), in agreement with older studies suggesting that antidepressant drugs (ADs), and in particular those acting on the serotonergic system, reduce the neurological disabilities occurring after stroke (Cramer, 2011; Mead et al., 2012).

Because fluoxetine might eventually become a first line treatment for stroke neurological symptoms, our goal here was to review, at physiological, cellular and behavioral levels, how SSRIs could exert therapeutic effects on the various facets of stroke.

Stroke is defined by the interruption of blood perfusion to a part of the brain. This cuts off the oxygen and nutrients supplies, and thereby causes damages to brain tissues. The majority of strokes is ischemic (85% are due to the occlusion of a blood vessel by a thrombus or an embolus), while a minority of them is hemorrhagic. Stroke is a serious neurological disease, particularly common and disabling. It affects 22 million people worldwide each year, and this number is increasing because of the aging population (IST-3 collaborative group et al., 2012). It is the second cause of death worldwide after cardiovascular diseases, being responsible for 9.7% of deaths in 2004 and this is expected to reach 12.1% in 2030 (Mathers et al., 2009). In addition, stroke is the leading cause of acquired disability in adults and is the second cause of dementia after Alzheimer's disease (Acheampong & Ford, 2012). Stroke diagnosis needs to be done as early as possible to limit its consequences and implement an appropriate treatment. The phrase "time is brain" emphasizes that nervous tissue is rapidly lost as a stroke progresses, since a typical patient loses 1.9 million neurons each minute stroke is untreated (Saver, 2006). Sudden neurological symptoms rapidly progressing over a few minutes or a few hours may be signs of a stroke. The most common stroke symptoms include hemiparesis that may involve face, arm and leg, hemisensory disturbances, gaze deviation, language disorder (dysphasia) and visuospatial neglect.

Risk factors for ischemic stroke include age, the most powerful factor (the risk of stroke doubles every decade after 55 years), gender (prevalence is higher in men), ethnic background, and family history. Modifiable risk factors are represented by hypertension, which is the major risk factor, heart diseases (atrial fibrillation being the most important),

smoking, diabetes and hyperlipidemia. Other possible modifiable risk factors are obesity, diet, physical activity, alcohol consumption, contraception, and hormone replacement therapy (Goldstein et al., 2011). Among psychological risk factors, a depressive mood may predispose to a stroke, in particular an ischemic stroke (May et al., 2002; Ohira et al., 2013). Metabolic changes characteristic of depressive patients may underlie this increased propensity for stroke. Depressed individuals very often have increased autonomic sympathetic activity that may lead to increased platelet activity. Furthermore, a hallmark of depression is the excessive secretion of glucocorticoids that itself promotes conventional stroke risk factors, such as hypertension. Elevated glucocorticoids are also known to decrease neurogenesis and trophic factors, which as we will see in section 3.2, may be stroke recovery mechanisms. Interestingly, depression and anxiety are associated with exaggerated serotonin-mediated platelet activation (Zafar et al., 2010), but patients treated with SSRIs may be protected against this risk factor, as 5-HT uptake inhibition have an inhibitory effect on platelet activation by decreasing 5-HT content inside platelets and thereby reducing platelet plug formation (Geiser et al., 2011; Hergovich et al., 2000).

Beyond a risk factor, depression is also known as a main consequence of stroke. The psychological distress reactions generated by the stroke itself may explain this late emotional deficit (Gainotti et al., 1997). Nevertheless, many authors insist instead on biological causes. Depression occurs more often in the stroke population than in patients presenting similar levels of disability from other diseases (Folstein et al., 1977). Post-stroke depression could be the result of a brain lesion in a region regulating emotions such as the prefrontal cortex (Terroni et al., 2011). However, whether an augmented risk of depression does exist after a stroke in a specific brain location rather than another remains a matter of debate (Carson et al., 2000; Narushima et al., 2003). In any case, lesions in areas such as the prefrontal cortex should have an important impact through their projections on monoaminergic systems. These systems and the neural circuits regulating mood and anxiety might suffer from a generalized damage to the brain vasculature, as first claimed in the “vascular hypothesis” of depression (Taylor et al., 2013). In contrast to post-stroke depression, which involves an acute and focal lesion, vascular depression would be the consequence of chronic small ischemic lesions to frontal and subcortical circuits regulating emotion and cognition. It is particularly fitted to explain late-onset depressions occurring after age 50. In old depressed patients who have experienced a brutal mood transformation, symptoms are more likely accounted by a biological dysfunction than traits and personality. Imaging studies have revealed abnormally of the white matter (white matter hyperintensities) in several brain areas of depressed patients, including the prefrontal cortex (Tupler et al., 2002). These small infarcts largely asymptomatic at first could eventually trigger a major depression, dementia or the occurrence of a larger ischemic stroke, itself leading to post-stroke depression. Note that fluoxetine and other SSRIs are well-tolerated first line agents to treat late-life depression, whether or not associated with stroke (Solai et al., 2001).

We will argue in the present review that fluoxetine is a pleiotropic drug for stroke treatment. Putative mechanisms explaining the beneficial effects of fluoxetine are multiple and may explain as much the therapeutic effects on motor functions as those on mood and cognition. First, SSRIs are known to have neurotrophic effects by acting on the production of growth factors, such as the brain derived nerve growth factor (BDNF) and other processes such as hippocampal neurogenesis. Migration of new neurons to damaged brain areas have been shown to occur after stroke (Wiltout et al., 2007). Both neurogenesis and angiogenesis

in stroke areas appear to be facilitated by SSRI treatments (Espinera et al., 2013). Second, SSRIs may have neuroprotective effects by reducing inflammation *via* an action on cytokines and by repressing microglia activation, and third, they might be acting on vascular and haemostasis parameters immediately after stroke (Geiser et al., 2011; Ofek et al., 2012). Finally, on the psychological sphere, the antidepressant and anxiolytic effects of chronic SSRI may help stroke recovery by attenuating a cascade of distress related processes.

1.1 The Neurovascular Unit and the Cerebral Blood Flow

To better understand brain damages caused by stroke, we need to consider primary events taking place at the blood brain barrier (BBB). The BBB regulates the entry of nutrients and drugs into the brain from circulating blood. As shown on figure 1, the BBB is composed of endothelial cells, a basement membrane, pericytes and astrocytic feet. All these elements are necessary to control exchanges between the blood and the brain parenchyma. Together with neurons and glial cells, they form a functional unit called the neurovascular unit.

Changes in the integrity of the BBB are central to the pathophysiology of cerebral ischemia. Ischemic stroke can disrupt the neurovascular unit at both the structural and functional levels, which leads to abnormal leaks across the BBB. Models of cerebral ischemia revealed how expression of both inter-endothelial junction proteins (such as occludin) and components of the basal membrane (such as collagen IV and laminin) can be decreased. Swelling of astrocytes leads to the detachment of their microvascular endfeet (figure 1). Among the mechanisms underlying post-ischemic damage of the BBB, those involving metalloproteinases (MMPs) play a major role by degrading the extracellular matrix and interendothelial junctions. These proteolytic enzymes expressed by endothelial cells, microglial cells, neurons, astrocytes and infiltrating inflammatory cells are involved in brain remodeling after cerebral ischemia (section 1.4).

Fluoxetine may inhibit ischemia-induced cell death and cognitive impairment, by preventing BBB disruption indirectly *via* its action on astrocyte / microglial activation and inflammation (Lee et al., 2014). It may also possibly act directly at the level of the neurovascular unit itself because expression of the 5-HT transporter (5-HTT) mRNA has been detected in cultured immortalized rat cerebral endothelial cell lines (Brust et al., 2000). This transporter appears to be localized on both the luminal and the abluminal sides of brain capillaries (figure 1; Wakayama et al., 2002).

Following an ischemic insult, there is a persistent microvascular constriction that results in part from pericyte contraction (Liu et al., 2012; figure 1). Post-stroke vasospasms and BBB dysfunctions also involve blood vessels composed of vascular smooth muscles regulating cerebral blood flow through innervation from noradrenergic, GABAergic, cholinergic and serotonergic neurons (Hawkins & Davis, 2005). Both *in vitro* and *in vivo* studies have shown that 5-HT act not only as a synaptic neurotransmitter in the central nervous system, but also as a potent vasoactive agent of the brain vasculature. 5-HT nerve fibers have been observed through the use of an antibody to tryptophan hydroxylase (the 5-HT synthesis enzyme), in major cerebral arteries and small pial vessels. These projections appear to be either of central origin, from the rostral raphe nucleus, or of peripheral origin, from the superior cervical ganglion (Bonvento et al., 1991). Because of its localization in key vascular targets, 5-HT is thought to play a pivotal role in coupling cerebral blood flow to metabolism. It decreases

oxygen and glucose consumption, depresses electrical activity in the cortex and reduces cerebral blood flow, mainly when the BBB is disrupted (for a review see Bonvento et al., 1991). Heterogeneous post-synaptic receptors (5-HT₁, 5-HT₂, 5-HT₇, ...) are involved in the vasomotor effects of 5-HT depending on the species, anatomically distinct vessels and even different segments of the same vessel (Bonvento et al., 1991). Some of the vasoconstrictor effects of 5-HT in small cerebral arteries are mediated by 5-HT_{2A} receptors via voltage-dependent Ca²⁺ channels (Ungvari et al., 1999). Furthermore, activation of 5-HT₃ receptors on GABAergic interneurons can indirectly mediate either constriction or vasodilatation via neuropeptide Y or NO, respectively (Perrenoud et al., 2012). Finally, the inhibitory somatodendritic 5-HT_{1A} autoreceptors on 5-HT neurons of the raphe nuclei could also play a role in increasing cerebral blood flow (Bonvento et al., 1997; Marco et al., 1999). Activation of inhibitory autoreceptors, as occurring when extracellular 5-HT concentration is increased by an SSRI, may thus decrease the postsynaptic receptor-mediated vasoconstrictor effect of 5-HT.

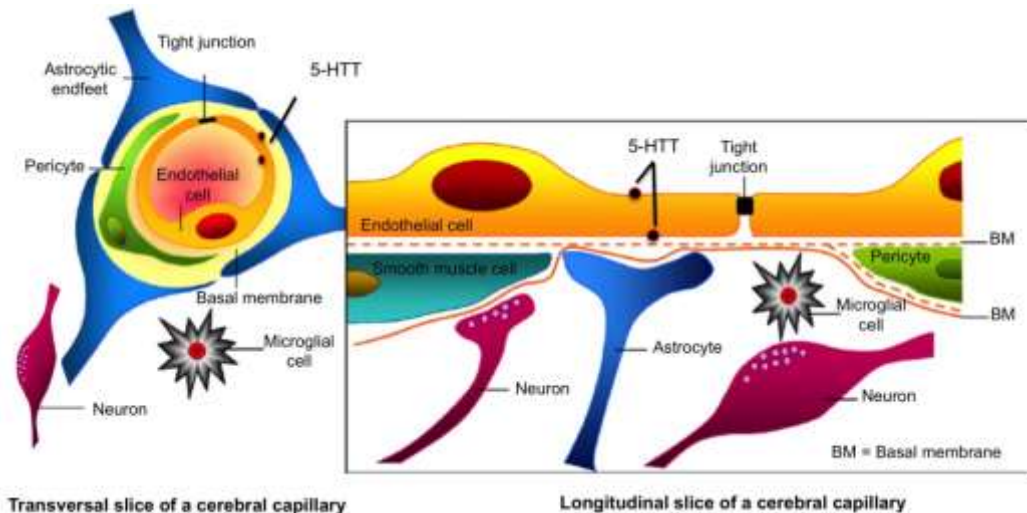


Figure 1. The principal elements constituting the neurovascular unit.

Endothelial cells form a continuous layer at the luminal surface of the capillary. They limit the permeability of the barrier via four defense levels: 1) interendothelial tight and adherens junctions that greatly reduce the paracellular flux through various proteins such as the tight junction protein occludin, 2) few pinocytotic vesicles forming a transcellular barrier, 3) enzymatic barriers and 4) endothelial cells expressing a large number of efflux transporters that allow efflux of toxins and drugs. Some of them participate to a certain extent to the inactivation of several neurotransmitters, including glutamate, GABA, noradrenaline and, in particular, 5-HT via the 5-HTT localized on both the luminal and abluminal sides. It is noteworthy that endothelial cells also express endothelial NO synthase (eNOS) exerting endothelium-dependent vasodilatation, platelet aggregation inhibition and anti-oxidant effects contrasting with the deleterious action of the inducible NOS (iNOS) triggered during inflammation. *The basal membrane* is an extracellular matrix on which is based the cerebral endothelium. It is composed of various proteins such as the cell adhesion molecule laminin and collagen IV. *Astrocytes* form a link between the endothelium and neurons. The astrocytic endfeet cover over 99% of the surface on the basement membrane of capillaries and microvessels. *Microglial cells* are also found in the perivascular space. They play a very important immunological role since they are the resident macrophages of brain

tissue. *Pericytes* are poorly differentiated contractile cells spreading around the capillary wall. Drowned in the basement membrane, pericytes cover the endothelium and provide structural support to blood vessels. Pericytes regulate brain capillary blood flow through contraction and relaxation (the figure was modified from Abbott et al., 2010).

1.2 Stroke and Cerebral Ischemia

Haemostasis, that prevents blood loss when vessels are damaged, is a vital defense process. It involves activation and adhesion of platelets, and subsequently fibrin matrix and blood clot formations. It can pathologically occur, however, in absence of bleeding, such as in stroke. The majority of strokes are ischemic, that is - due to the occlusion of a blood vessel by a thrombus or an embolus. A « haemostatic » plug interrupts blood flow, thereby causing cerebral ischemia and death of brain tissue downstream (infarction). In focal ischemia, occlusion of a cerebral artery occurs after migration of an embolus of extracerebral origin or by the formation of a thrombus *in situ*. The latter emerges in reaction to atherosclerosis or other vascular dysfunctions. The most frequently occluded cerebral artery is the middle cerebral artery (MCA). Ischemic areas are divided into the core area, where the fall in cerebral blood flow is severe enough to causes irreversible tissue loss (i.e., cell death), and the penumbra, which has residual blood flow and consists of cells initially not showing alterations of morphological integrity, but having impaired functions. The latter cells remain alive for a few hours and are the target of neuroprotective and reperfusion treatments.

Most of the preclinical studies on cerebral ischemia, in particular those about the effects of SSRIs (section 2 and 3), derive from animal models of cerebral ischemia that reproduce focal ischemia. They consist in MCA occlusion (MCAo), for instance by a microclip or by introducing a nylon filament to block blood flow. In other models, occlusion is carried out by injecting blood clots or thrombin into the artery. *In vitro* models are performed on cell cultures, more rarely on cultured brain slices. Different cell types can be cultured (neurons, astrocytes, oligodendrocytes, microglia, endothelial cells; see figure 1) alone or in co-cultures, such as astrocytes / neurons co-cultures. Cells are exposed to deprivation of either oxygen or glucose reproducing *in vitro* ischemic conditions. They can also be subjected to conditions that mimic some other events of cerebral ischemia: oxidative stress or glutamate-induced excitotoxic stress. These studies revealed the important consequences of ischemia on brain cells, as detailed in the following section.

1.3 Cellular Consequences of Brain Ischemia: Glutamate-Induced Excitotoxicity, Oxidative Stress and Inflammation

Following cerebral ischemia, the decrease in cerebral blood flow reduces input of energy substrates, oxygen and glucose. Energy depletion notably disrupts the function of the Na^+/K^+ ATPase, which consumes under physiological conditions, 70% of the brain energy resources. The resulting loss of ionic gradients triggers neuronal depolarization and increases the release of neurotransmitters, particularly the main excitatory amino acid glutamate. This leads to an accumulation of glutamate into the extracellular medium and excessive stimulation of its receptors, including the NMDA (N-methyl-D-aspartate) receptor. These receptors operating

channels, widespread in the central nervous system, are permeable to Ca^{2+} . The glutamate-mediated increase in intracellular Ca^{2+} concentration stimulates proteases, lipases, endonucleases, and neuronal NO synthase (nNOS), all contributing to the so-called excitotoxic cell death (Doyle et al., 2008; Turner et al., 2013).

In parallel with the huge increase in glutamate extracellular concentration, there is also a dramatic increase in 5-HT levels associated with brain ischemia (Kanthan et al., 1996; Nagao et al., 1995). Similarly to glutamate, 5-HT is associated with the negative consequences of brain injuries, such as brain edema formation and BBB disruption, because these consequences are attenuated by either 5-HT biosynthesis inhibition or 5-HT antibodies (Sharma et al., 2007). Furthermore, following BBB disruption, increasing 5-HT levels produces marked decreases in blood perfusion in numerous brain areas (Grome & Harper, 1983). Acute 5-HT reuptake inhibition with citalopram was shown to reduce ischemia-induced 5-HT hypermetabolism (Nagao et al., 1995).

Under physiological conditions, cells produce various free radicals, in particular through their mitochondrial respiratory chain. There is normally a dynamic equilibrium between the formation of free radicals and their disposal by endogenous enzymatic antioxidant systems (superoxide dismutase, glutathione peroxidase) and non-enzymatic processes (glutathione, vitamin E, vitamin C, ...). After brain ischemia, mitochondrial respiratory chain dysfunction and post-ischemic activation of various enzymes, such as iNOS and COX, leads to overproduction of free radicals, especially reactive oxygen species (ROS) including superoxide anion ($\text{O}_2^{\cdot-}$), hydroxyl radical ($\cdot\text{OH}$) and nitric oxide ($\cdot\text{NO}$). Under ischemic conditions, depletion of the L-arginine substrate and/or the co-factor BH₄, required for coupling two functional eNOS subunits, generates excessive ROS in endothelial cells leading to eNOS uncoupling. In an uncoupled state, eNOS produces harmful $\text{O}_2^{\cdot-}$ instead of the beneficial endothelial NO (which exerts vasodilation and antithrombotic effects). Once generated, $\text{O}_2^{\cdot-}$ activates several stress-sensitive pathways and scavenges NO to produce ONOO⁻, a highly reactive pro-oxidant that elicits direct neurotoxic effects (Srivastava et al., 2012). This ONOO⁻ causes DNA damages that hyperactivate the poly(ADP-ribose)polymerase (PARP), a DNA repair enzyme known to be deleterious when it is over activated during cerebral ischemia (Haddad et al., 2006; Szabó & Dawson, 1998). Post-ischemic overproduction of ROS also exceeds the capacity of endogenous antioxidant systems causing membrane lipid peroxidation, protein oxidation and cell death (Pradeep et al., 2012).

Post-ischemic oxidative stress and the resulting necrotic cells trigger the production of inflammatory cytokines. These initial events lead to the activation of microglial cells that in turn, produce glutamate, cytokines and chemokines in large amounts, which themselves causes the expression of adhesion molecules by endothelial cells. This triggers the adhesion of circulating inflammatory cells, *i.e.* leukocytes (neutrophils, lymphocytes and monocytes) to the endothelium and their subsequent infiltration into the brain parenchyma. Infiltrated leukocytes and microglial cells express and release various inflammatory mediators (cytokines, MMPs, iNOS, ...; Ronaldson & Davis, 2012). High levels of neurotoxic mediators, such as high concentration of NO and peroxide, inflammatory cytokines and proteases are involved in the post-ischemic disruption of the BBB (Yenari et al., 2006). We will see that fluoxetine could target many of these damaging processes (see section 2.3).

1.4 Brain Remodeling after Cerebral Ischemia

Post-ischemic disruption of the BBB notably results from a rise in MMPs, proteolytic enzymes that have the ability to remodel the extracellular matrix. These enzymes are necessary for inflammation and wound repair by cleaving several substrates including growth factors, cytokines, cell surface receptors and cell adhesion molecules. Studies have demonstrated the induction of several MMPs (MMP-2,-3,-7,-9) following cerebral ischemia (Jin et al., 2010; Sandoval & Witt, 2008). Post-ischemic oxidative stress is implicated since MMPs can be strongly activated by free radicals. MMPs are secreted by astrocytes, endothelial, microglial and neuronal cells, and by neutrophils infiltrated in the damaged brain parenchyma (Lakhan et al., 2009).

MMPs can also cleave and activate proneurotrophins, leading to mature forms of growth factors such as BDNF, regulating neuronal survival and synaptic plasticity *via* its tyrosine kinase (TrkB) receptor (Hwang et al., 2005; Lee et al., 2001). BDNF levels are increased at and around the lesion site of an ischemic stroke, through a timely secretion from neuronal and non-neuronal sources (endothelial cells, astrocytes and microglia; Béjot et al., 2011). Together with growth factors secretion, stroke induces brain repair mechanisms by stimulating new neuronal formation. Neurogenesis is triggered by neural stem cells in the dentate subgranular zone of the hippocampus and the subventricular zone lining ventricles adjacent to this area. New neurons migrate within the hippocampus and other areas of the brain such as the striatum, and this migration occurs in close apposition to blood vessels. The vasculature thus plays an essential role in this migration since neurogenesis was shown to occur in parallel with hypoxia-induced angiogenesis (Thored et al., 2007). Furthermore, in addition to growth factors (such as BDNF), various mediators of the ischaemic-hypoxic response (erythropoietin) as well as glutamate intervene at different steps to regulate neural progenitor proliferation, migration and survival in the post-ischemic brain (Kokaia & Lindvall, 2003; Wiltout et al., 2007). This cell proliferation appears important since irradiation-induced ablation of neurogenesis exacerbates behavioral deficits after cerebral ischemia (Raber et al., 2004). Enhancement of serotonergic transmission by chronic fluoxetine might also boost brain remodeling after stroke as it stimulates BDNF secretion and neurogenesis (see section 2.4).

2. EXPERIMENTAL AND CLINICAL STROKE THERAPEUTIC AND THE ACUTE EFFECTS OF FLUOXETINE

2.1 Drugs Acting on Haemostasis

Thrombolysis with the recombinant tissue plasminogen activator (rt-PA, alteplase) remains the only pharmacological treatment currently available for acute ischemic stroke. Endogenous tPA converts inactive plasminogen to plasmin, which in turn degrades the fibrin matrix. Treatment with rt-PA thus aims to lyse the thrombus to allow recanalization of clotted artery, and thereby permits tissue reperfusion. Rt-PA is the only thrombolytic agent proved clinically effective when administered 4.30 hours after symptoms onset. However, to date, only 3-4% of patients suffering from an acute ischemic stroke are treated with intravenous rt-

PA. The barriers to thrombolysis are the delay in recognizing stroke symptoms at hospital arrival and the multiple contraindications to rt-PA administration. The use of rt-PA is indeed not without risk since it has been shown to enhance the occurrence of symptomatic hemorrhagic transformations *via* multiple factors, including inflammation (Haddad et al., 2013). Furthermore, early reperfusion after intravenous rt-PA can effectively be established in fewer than 40% of treated stroke patients (von Kummer & Hacke, 1992).

Interestingly, one possible mechanism through which fluoxetine exerts a beneficial action in stroke recovery is *via* an action on blood coagulation and fibrinolysis. 5-HTT plays a role in the activity of platelets: these anucleated cells are unable to synthesize 5-HT, but they actively uptake it from the plasma via 5-HTT. Endothelium damage, caused among other by arteriosclerosis triggers platelet activation and the content of platelet granules, including 5-HT, is released thereby enhancing the Ca^{2+} -dependent amplification of platelet activation. Although 5-HT *per se* does not activate platelets, it enhances platelet activation by factors such as ADP, adrenaline and thrombin via 5-HT_{2A} receptors (Li et al., 1997). Note that the latter receptors are also expressed on the vascular walls to exert, concomitantly with haemostasis, vasoconstriction (section 1.1). Interestingly, anxious and depressed patients treated with serotonergic ADs displayed enhanced fibrinolysis; these drugs thus prevent a basal state of enhanced blood coagulation produced by platelets hyperactivity in these patients (Geiser et al., 2011). Furthermore, 5-HT appears to have a crucial role in the formation of a subpopulation of platelets, the coated-platelets dually activated by collagen and thrombin, which may lead to a robust pro-thrombotic state, and accordingly, individuals treated with SSRIs have a reduced proportion of these platelets (Prodan et al., 2007). By blocking uptake and storage of 5-HT into platelets via 5-HTT, fluoxetine may prevent the platelet aggregation and coagulation cascade. Clinical studies that have investigated the existence of an association between chronic SSRI treatments and stroke did not detect, however, a reduced risk (de Abajo, 2011). Nevertheless, it is conceivable that acute fluoxetine administration after a stroke prevents reocclusion contributing to chronic small ischemic lesions. However, similarly to rt-PA, SSRIs might also increase the risk of brain hemorrhage, although this risk appears to be moderate (Hackam & Mrkobra, 2012).

2.2 Drugs Promoting Vasodilation and Blood Reperfusion

Recanalization with rt-PA is necessary, albeit not sufficient to allow full blood reperfusion of brain tissue via microvessels. Reperfusion is, in fact, a better predictor of stroke recovery than recanalization (Soares et al., 2010). Following cerebral ischemia, calcium not only triggers several deleterious enzymatic cascades (section 1.3), but also induces vasomotor contraction through voltage dependent-gated channels on microvessels smooth muscles, leading to vasospasms (Cipolla et al., 2014). Based on preclinical studies, the vasodilation effect of calcium antagonists, combined with their neuroprotectant action *via* an attenuation of intracellular Ca^{2+} concentrations, was hypothesized to confer therapeutic efficacy. Unfortunately, calcium antagonists, such as nimodipine, failed to show clinical benefits in several trials on ischemic stroke patients (Zhang et al., 2012).

Other drugs might be more suitable to release the vasospasms. Interestingly, fluoxetine might be such an agent because its acute administration was shown to produce vasodilation of cerebral arterioles and to ameliorate reperfusion of the ischemic brain tissue. Furthermore, it

was shown to prevent the vasoconstrictor response triggered by either 5-HT or by a calcium channel opener in vascular smooth muscle of small cerebral arteries (Ungvari et al., 1999). This may occur through the release of endothelial NO since a eNOS antagonist blocked this vasodilation effect (Ofek et al., 2012). Apparently this is a 5-HT-independent effect because it was not blocked by the non-selective 5-HT antagonist methysergide (Ofek et al., 2012). Nevertheless, whether selective 5-HT₃ receptor antagonists would alter the effect of fluoxetine needs to be assessed as 5-HT₃ receptors also mediate vasodilatation through NO (Perrenoud et al., 2012). According to Ofek et al. (2012), the indirect effect of fluoxetine involves rather an action of acetylcholine released from parasympathetic fibers and binding of this neurotransmitter on muscarinic receptors of endothelial cells (Ofek et al., 2012). This results into the release of calcium from intracellular stores in these cells and facilitates binding of calmodulin to eNOS that then produces NO from L-arginine. NO subsequently diffuses to vascular smooth muscles to induce vasodilation. Fluoxetine at low doses may also enhance noradrenaline release from cerebral perivascular sympathetic nerves and may cause, through β_2 receptors, a NO-mediated vasodilation *via* axo-axonal mechanism, independently of the endothelium (Chen et al., 2012).

2.3 Drugs Preventing Cellular Damages

As we have seen in section 1.3, neuroinflammation is one of the most important mechanisms leading to brain cell damages. A major event at the cellular level leading to the inflammation cascade is oxidative stress. Therefore, preventing oxidation stress appeared, at least in several experimental stroke models, as one of the most promising pharmacological target. Several anti-oxidant compounds have been tested clinically, including NXY-059, which unfortunately was revealed ineffective in large trials on acute ischemic stroke (Diener et al., 2008). Another important process in inflammation is immune cells adhesion and infiltration in the infarct area. Enlimomab, developed to prevent these processes, did not show efficacy in a clinical trial and had even worsen stroke outcomes (Enlimomab Acute Stroke Trial Investigators, 2001). Among the plethora of strategies researchers had hope would be beneficial in human stroke, there is also the prevention of excitotoxicity with glutamate antagonists, but these drugs also failed to fulfill expectancies (Muir, 2006).

Fluoxetine and other SSRIs shown to be clinically effective have, in turn, an interesting pattern of action at some of these same cellular processes. First, fluoxetine may inhibit cell death after stroke by blocking BBB disruption. In an experimental model of global ischemia, subchronic treatment with fluoxetine inhibited MMP-2 mRNA expression, reduced MMP-9 activity and attenuated the loss of laminin and occludin, substrates of MMPs (Lee et al., 2014). As visualized with occludin antibodies, the vascular network integrity was restored after fluoxetine treatment in ischemic animals, and this probably explains the decrease in immune blood cell infiltration concomitantly observed (Lee et al., 2014; Lim et al., 2009). Second, recent *in vivo* studies as well as studies on cultured cells indicate that fluoxetine can suppress microglial and astrocyte activation resulting from ischemic insults (Dhami et al., 2013; Lee et al., 2014; Lim et al., 2009). These therapeutic-like effects were correlated with improvements both in terms of motor activity and memory, and in terms of infarct volume and apoptotic cell death (Lee et al., 2014; Lim et al., 2009). Importantly, treatment with high acute doses of SSRIs appears particularly effective, contrary to chronic SSRI treatments that failed to induce

therapeutic effects at this level (Jolkkonen et al., 2000; Lim et al., 2009; Windle & Corbett, 2005; Zhao et al., 2005). In a co-cultured cell study, oxygen-glucose deprived cortical neurons showed greater survival in presence of activated microglial cells when they were pre-treated with fluoxetine, but not with other types of ADs such as tricyclic antidepressants, (TCA) and monoamine oxidase inhibitors (MAOI) (Dhami et al., 2013). Neurons would be protected under fluoxetine apparently because glutamate excitotoxicity resulting from microglial activation is reduced (Dhami et al., 2013). The primary mechanism accounting for the decreased glutamate release from activated microglia in presence of fluoxetine remains unclear, although interactions between serotonergic and glutamatergic systems are known to occur in the brain (Drago et al., 2011).

Fluoxetine not only influences immune cells infiltration and microglial activation, but also the capacity of these cells to produce proinflammatory agents. Protein and mRNA expression of inflammatory mediators such as TNF- α , IL-1 β , IL-6, COX-2 and iNOS and several chemokines triggered by ischemia are markedly decreased by fluoxetine (Lee et al., 2014; Lim et al., 2009). This is associated with an inhibition of NF- κ B, a transcription factor that controls DNA elements involved in cellular responses to cytokines (Lim et al., 2009).

Although drugs having a primary action on oxidation/inflammation, such as NXY-059 and enlimomab, failed to show clinical efficacy (Diener et al., 2008; Enlimomab Acute Stroke Trial Investigators, 2001; Muir, 2006), it remains possible that fluoxetine's peculiar pattern of action on glutamate excitotoxicity and inflammation explain its therapeutic action in stroke patients.

3. ANTIDEPRESSANT-LIKE EFFECTS OF FLUOXETINE IN POST-STROKE DEPRESSION

Preclinical research interest has recently shifted toward the long-term cognitive and emotional deficits of stroke. About one-third of patients display anxio-depressive symptoms after stroke onset (Carota et al., 2005; Hackett et al., 2014). Post-stroke depression is a major problem as it slows recovery, predicts poorer functional outcomes and increases the risks of mortality, including suicide (Kronenberg et al., 2014). Depression itself, which as we have seen increases the risks of stroke (May et al., 2002; Ohira et al., 2001), could be associated with BBB barrier disruption, eNO uncoupling, MMPs induction (Najjar et al., 2013; Taylor et al., 2013; Yoshida et al., 2012) and the generation of chronic small ischemic lesions (Dieguez et al., 2004). It is thus becoming urgent to adequately model at the preclinical level this stroke-depression association, especially with the prospect that drugs with antidepressant properties, SSRIs, might become the first pharmacological treatment for both the subacute and the chronic phases of stroke recovery (Kronenberg et al., 2014).

Behavioral models commonly used to rapidly screen for ADs such as the forced swim test (FST) may not be adequate. Cerebral ischemia in rodents is very often associated with motor hyperactivity resulting in decreased immobility in the FST, an antidepressant-like effect. In fact, the SSRI fluvoxamine and cerebral ischemia were shown to have additive antidepressant-like effects in the FST (Deplanque et al., 2011). Modeling symptoms of depression such as anhedonia, i.e., the inability to experience pleasure, provides more interesting information. It has been reported that MCAo can decrease by itself mice sucrose

consumption, and this was associated with a degeneration of mesolimbic dopaminergic pathways involved in reward behaviors (Kronenberg et al., 2012). Long-term treatment with the SSRI citalopram prevented anhedonia and the neurodegeneration occurring after cerebral ischemia (Kronenberg et al., 2012). MCAo alone might sometimes be insufficient to trigger anhedonia and it is often combined with a protocol of chronic mild stress to produce a depression-like state (Wang et al., 2009). Here again, at various time windows, several weeks after MCAo, chronic SSRI treatment was shown to prevent the decrease in sucrose consumption (Wang et al., 2009; Wang et al., 2010; Wang et al., 2010). As in patients, cerebral ischemia sometimes produces in rodents long term anxiety and spatial cognitive impairments that are likewise corrected by long term fluoxetine or citalopram treatments (Kronenberg et al., 2012; Li et al., 2009).

The behavioral deficits modeling post-stroke depression are associated with decreased 5-HT levels in the frontal cortex and the hippocampus (Ji et al., 2014). Serotonergic projections to brain areas involved in emotional and cognitive functions are most likely crucial for stroke recovery. Destruction of hippocampal serotonergic fibers with the toxin 5,7-dihydroxytryptamine aggravates ischemic neuronal damages (Nakata et al., 1997). Although it is beneficial to limit, not abolish, serotonergic mechanisms at the initial phase of a stroke (see section 2), on the long term, the enhancement of serotonergic transmission (as explained below) would rather favor brain remodeling and antidepressant-like behavioral effects.

3.1 Chronic Fluoxetine Increases Post-Synaptic 5-HT_{1A} Receptor-Mediated Neurotransmission

Microdialysis studies have clearly shown that 5-HTT blockade rapidly increases 5-HT extracellular concentration following either systemic SSRI injections or local SSRI injections into a brain area. However, when 5-HTT is blocked locally prior to a systemic SSRI injection, the latter induces a decrease of extracellular 5-HT concentration. This is surprising but explained by a decrease release of 5-HT resulting from activation of negative feedback mechanisms involving inhibitory autoreceptors (Auerbach et al., 1995; Hervás & Artigas, 1998). Acutely, SSRI administration decreases the spontaneous activity of serotonergic neurons, and this can obviously not explain their long-term therapeutic effect in post-stroke depression. Following weeks of treatment with these same SSRIs, the basal firing rate of neurons is re-established because of somatodendritic 5-HT_{1A} autoreceptors desensitization. A majority of authors consensually admit that this desensitization is at the core of their therapeutic effect in depression (Blier & de Montigny, 1994). Some other studies have also shown that inhibitory terminal 5-HT_{1B} or 5-HT_{1D} autoreceptors, regulating 5-HT release, might also become desensitized after chronic ADs (Chaput et al., 1986; Newman et al., 2004). Beside autoreceptors, other desensitizations occurring at post-synaptic 5-HT_{2A/2C} receptor subtypes might further explain the anxiolytic effect of SSRIs (Martin et al., 2014; Mongeau et al., 2010; Weisstaub et al., 2006). Activation of brain 5-HT_{2C} receptors during stress may indirectly inhibit 5-HT released at post-synaptic 5-HT_{1A} receptors and thereby limit the capacity of an animal to cope with stress (Mongeau et al., 2010). Similarly to other ADs (TCA, MAOIs), SSRIs are known to increase post-synaptic 5-HT_{1A} neurotransmission. Chronic SSRIs do not change the sensitivity of post-synaptic 5-HT_{1A} receptors, but rather

increase the amount of 5-HT released per action potential acting at these receptors (Chaput et al., 1991).

In the anhedonia model of post-stroke depression, animals displayed reduced 5-HT_{1A} receptor protein and mRNA levels in the hippocampus, and these changes were reversed by a citalopram treatment (Wang et al., 2009). In keeping with the view that increasing post-synaptic 5-HT_{1A} transmission leads to favorable outcomes in stroke, activation of 5-HT_{1A} receptors prevents cAMP-dependent protein kinase A phosphorylation of a NMDA receptor subunit contributing to neuronal death in the post-ischemic brain (Salazar-Colocho et al., 2007), and 5-HT_{1A} receptors may have the ability to decrease the excessive release of glutamate induced by ischemia (Semkova et al., 1998). Furthermore, differential activation of either 5-HT_{2C} or 5-HT_{1A} neurotransmission increase and decrease, respectively, cellular damages after cerebral ischemia (Torup & Diemer, 2000), while impairments of glucose uptake and hippocampal electrical activity after cerebral ischemia were both prevented by either 5-HT_{1A} receptor agonists or by 5-HT_{2A/2C} receptor antagonists (Shibata et al., 1992).

3.2 Chronic Fluoxetine Alters Trophic Factors and Cell Proliferation

Contrary to post-stroke depression, there are few neuro-anatomical correlates of major depression itself. Nevertheless, many clinical imaging studies indicate reduced hippocampal volume in depressed patients and this degeneration occurs as a function of stress chronicity and depression duration (McKinnon et al., 2009). ADs, whether or not they have a primary action on 5-HT, may induce the production of trophic factors, including BDNF. Stimulation of TrkB by BDNF triggers intracellular cascades inducing trophic effects in the hippocampus and other brain regions. The fact that tissue levels of BDNF are decreased in the brain of suicide patients and in the serum or the plasma of depressed patients (Lee & Kim, 2010) may explain, at least in part, hippocampal volume loss in these patients. Furthermore, there is a strong relationship between prior serum BDNF levels and the development of post-stroke depression (Li et al., 2014). Interestingly, the progressive fall in hippocampal BDNF levels occurring in animal models of transient ischemia was prevented by either fluoxetine or escitalopram treatment (Kim et al., 2007; Lee et al., 2011). Beyond the hippocampus, the beneficial effect of BDNF secretion following chronic SSRI treatment extends to sensory-motor cortical areas (Espinera et al., 2013; Maya Vetencourt et al., 2008). Post-stroke depression-like behaviors and the reduction in 5-HT neurotransmission are both associated with depletion of other trophic factors, such as the fibroblast growth factor (FGF-2) known to protect cerebral tissue from ischemia by activating anti-apoptotic proteins (Ji et al., 2014).

Another important recovery process that could alter hippocampal morphology is neurogenesis. It is now well-known that 1) new neurons are normally generated in this area during the whole life, 2) a decrease in neurogenesis occurs during chronic stress, and 3) the latter can be corrected by all class of ADs (Gould, 2007; Malberg, 2004; Paizanis et al., 2007). Despite being a major stress, cerebral ischemia triggers hippocampal neurogenesis, but this neurogenesis is inversely related to ischemia severity (Winkelheide et al., 2008). Neurogenesis is a mechanism necessary to reinstate some functions after brain insults since its conditional ablation attenuates recovery of motor function (Sun et al., 2012). The depressive-like behaviors of MCAo rats submitted to chronic mild stress were shown to be associated with reductions of various ischemia-induced neurogenesis processes, including cell

proliferation, survival and neuronal differentiation (Wang et al., 2008). Chronic treatments with either fluoxetine or citalopram were shown to increase hippocampal neurogenesis in experimental models of stroke, and this was correlated with improvements of late emotional and cognitive deficits (Li et al., 2009; Wang et al., 2010). Interestingly, these post-stroke improvements in terms of neurogenesis and behavioral antidepressant-like effects were accelerated by co-administration of an antagonist at the inhibitory 5-HT_{1A} somatodendritic autoreceptors (Wang et al., 2010). Furthermore, chronic buspirone, a partial agonist at post-synaptic 5-HT_{1A} receptors (and effective as fluoxetine as an anxiolytic drug) also increase neuronal cells proliferation in the hippocampus (Campbell Burton et al., 2011; Grabiec et al., 2009).

The effect of SSRIs on neurogenesis and BDNF may be implicated in neurovascular unit repair in the peri-infarct area. Citalopram was shown to stimulate neuroblasts proliferation, to enhance cells migration from the hippocampus to the peri-infarct regions, to increase BDNF protein and to improve microvessels density in the damaged cortex (Espinera et al., 2013). Interestingly, there might be a causal relationship between angiogenesis and neurogenesis stimulation by SSRIs even in treated depressed patients that had not suffered a stroke (Boldrini et al., 2012)

4. CONCLUSION AND PERSPECTIVES

Clinical studies have confirmed the efficacy of ADs, mostly SSRIs, not only in post-stroke depression but also in alleviating cognitive and motor deficits occurring after a stroke. In view of the shortcomings in the development of new pharmacological treatments for stroke, these are by any standards exciting results. Nonetheless, as argued by Cramer (2011), many questions remain at the clinical level regarding therapeutic time windows, the prognosis and the range of stroke deficits that can effectively be treated with fluoxetine or other SSRIs. Furthermore, larger clinical trials should be designed based on accumulated knowledge at the preclinical level about fluoxetine's mechanism of action. Yet, we do not really know how SSRIs exert their therapeutic effect in stroke, although several hypotheses have been reviewed here. Blocking 5-HT uptake and storage into platelets may reduce platelet aggregation and the coagulation cascade leading to either less recurrent strokes or chronic small ischemic lesions. A second possibility is the indirect vasodilator effect of SSRI *via* endothelial NO that might help reperfusion of penumbra areas. Not only would SSRI counteract hypoperfusion, they would also prevent the post-stroke increase in BBB permeability through an action on MMPs at the basal membrane of the neurovascular unit. In these two cases, by either preventing 5-HT uptake or by indirectly increasing 5-HT autoreceptor-mediated negative feedback, acute SSRIs would reduce the increased 5-HT metabolism generated by ischemia. Acute high doses of SSRIs were also shown to decrease the cellular (microglial activation) and the molecular (cytokines, chemokines) actors of the inflammation cascade and this might be explained notably by 5-HT / glutamate interactions. One may wonder, however, how important any of these mechanisms are in explaining the therapeutic effect of fluoxetine. Nevertheless, at this point, it remains reasonable to assume that fluoxetine is a pleiotropic drug exerting action on distinct mechanisms that are evolutionary linked to the role of 5-HT in defense to acute injuries.

This contrasts with our traditional views about fluoxetine being an antidepressant medication. Post-stroke depression and cognitive decline are associated with decreased 5-HT transmission. It has long been argued that chronic ADs enhance 5-HT neurotransmission, stimulate the secretion of neurotrophic factors and facilitate neurogenesis in brain areas such as the hippocampus. These physiological effects have been associated with antidepressant-like behaviors in various animal models of anxious-depressive disorders as well as in models of post-stroke depression. SSRIs might have a dual benefit in stroke; at high acute doses, or after relatively short term treatments, they would reduce post-stroke 5-HT hypermetabolism, trigger anti-thrombotic, vasodilator and anti-inflammatory mechanisms, while on the long term, SSRIs would rather desensitize autoreceptor-mediated feedback mechanisms and enhance 5-HT transmission, thereby favoring brain repair processes through neurotrophic factors and cell proliferation. Clinical studies remain necessary to determine how various fluoxetine administration protocols differentially favor short and long term recovery mechanisms in stroke patients, and to which extent any of these effects are based on either neurovascular, neuroprotectant or antidepressant actions.

5. REFERENCES

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Chapter 64

CORRELATION BETWEEN EXECUTIVE AND MOTOR FUNCTION IN PATIENTS AFTER STROKE

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ABSTRACT

Executive functions refer to a number of cognitive processes that use and modify information from cortical sensory systems in the anterior and posterior regions of the brain, so they could alter and produce behavior and movement. These integrative functions, including motor and behavioral components are necessary for effective aimed actions that represent base for performing activities of daily living. The most important components of executive functions are selection of the goal, planning, inhibition of irrelevant responses and impulse, monitoring and control activities, as well as the evaluation of the action outcome. Attention control, working memory and temporal organized behavior, also stand out as a special executive functions.

Cognitive impairment after a stroke may be complex, particularly for the involvement of the cerebral hemisphere or the frontal lobes, and depending on the lesion, executive function could be more or less effective. The function of the walking is the most compromised motor function.

The subject of this study was to examine the correlation between executive functions, attention and walk disorders after a stroke and how that relation influences the recovery process and achievement of functional independence in patients. Within the aim of the research, the relation between the levels of organization activities, attention and walk disorders in patients after a stroke had been analyzed.

The examined sample consisted of 50 patients after a stroke, included in the rehabilitation process and 50 patients randomly chosen, matched by age and general characteristics, which in its medical history and neurological examination, had no symptoms of acute or chronic neurological disease. For the evaluation of executive functions, attention, cognitive state, the Tests for the evaluation of motor functions

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including the evaluation of all gait parameters and the Test for the evaluation of functional abilities have been used in the paper.

The received results show that the patients after stroke have a statistically important lower achievement at the Test for evaluation of attention ($p < .001$), a statistically important lower achievement at the Test of executive functions ($p < .001$), that 72% of examinees have a minor cognitive disorder, while 6% of the examinees have a major cognitive disorder after a stroke. The obtained results also show a statistically important lower efficiency in all examined motor functions, in the gait frequency ($p < .01$), gait speed ($p < .001$), the length of pace ($p < .001$), the motor leg index ($p < .001$), obstacle management and balance keeping ($p < .001$). The results confirm the existence of a statistically important difference in all examined variables between the two groups of examinees ($p < .001$). A statistically important difference in results of the right-sided and left-sided hemiparesis of the examined patients after the stroke had been noticed at executive functions ($p < .05$), while at motor functions the difference is of no statistical importance. Within the group of after-stroke patients there is a statistically important link between the level of cognitive damage and the examined parameters of walk ($p < .05$). Furthermore, within the group of after-stroke patients there is a statistically important link between the level of cognitive damage and functional ability ($p < .05$).

Keywords: Stroke, executive functions, cognitive functions, attention, walk, functional recovery

INTRODUCTION

A stroke as a widespread medical problem is for some time undergoing the different stages of the great interest of various experts who deal with this problem. In assessing the significance of this disease one should bear in mind that the stroke is one of the leading causes of functional disability.

According to the World Health Organization (WHO, 1980) stroke is a clinical syndrome of vascular etiology that is characterized by the sudden onset of focal or global cerebral deficit lasting more than 24 hours or ending in lethal outcome. World Health Organization data show that, despite diagnostic and therapeutic possibilities, stroke is the third most common cause of death in the world (right after cardiovascular disease and cancer) and the second most common cause of functional disability (Goldstein et al., 1989).

The data that indicates a high degree of functional disability, which ranges from light (35.8%), moderately severe (33.3%) and severe (30.9%), gives cause for concern (Peterea, 2009). Having in mind the current statistics, the role and importance of medical rehabilitation is quite understandable.

Cognitive deficit within the stroke has numerous significances. It is assumed that there is dependence in quality of motor recovery and improvement in functional status with the degree of preservation of cognitive function. It is important to do a functional assessment which includes an assessment of cognitive functions prior to the rehabilitation process of patients after a stroke.

Traditional cognitive assessment includes the use of tests for assessing the achievements and measurement of specific cognitive functions. Many of the tests are well standardized in the general population, while some of them are still developing, all in aim of finding the most sensitive tests for detecting subtle brain function disorders, of any kind of genesis.

In few recent decades many experts have been involved in the issue of cognitive deficits as a part of stroke, as well as the impact of existing connections with motor disorders, with an emphasis on walking function in patients after a stroke. Preservation of cognitive function is of particular importance for the rehabilitation of patients after a stroke. According to cognitive competence, a patient after a stroke during the rehabilitation process will be able to set a goal for himself and to assess the real possibilities of achieving that goal. The multiple cognitive impacts on walking and link between movement control and appropriate behavior during the walk had been observed. It is manifested by consciousness of the individual about destination, the ability to appropriately control limb movement that produce walk and the ability to move through often very complex environment, in order to successfully reach the desired destination. Up until recently, walk was considered mainly as an automatic motor task which requires minimal cognitive engagement on a higher level. However, growing evidence link changes in the ability to organize activities and attention to the present irregularities of walk (Seligman et al., 2008). One of the major goals of the rehabilitation process is to help the patient to achieve the highest possible level of functional independence, in which walk is the basic component of independent functioning. It is assumed that this approach to the rehabilitation can have a positive reflection on the course and outcome of rehabilitation treatment.

Therefore, guided by the experience of all those who have dealt with this problem, while adhering to all the rules in monitoring clinical characteristics of cognitive competence and functional recovery, we have reached the yet unexplored field that connects the executive function and motor impairment in patients after a stroke. If we have chosen a good path and contributed to a better understanding of this problem, we can be somewhat satisfied with what we have tried to do. Perhaps future research stimulated by this work and previous works in this field will be able to highlight the importance of this problem and clearly define the link between cognitive dysfunction and motor damage after a stroke, as well as to create new opportunities and make appropriate treatment programs and to implement them in the process of medical rehabilitation, fully supporting them.

Organization of Neuropsychological Functions Responsible for Planning and Organizing Activities - Executive Functions

Prerequisites for the development of planning and organizing activities lie in the complex functioning of the psychological functions represented in hierarchical and dynamically organized neuroanatomical systems. The theory of functional systems relies on the dynamic localization of higher mental functions. In accordance with this theory, the anatomical basis of cognitive functioning is consisted of three hierarchical functional blocks.

Luria (Luria, 1983) considers that for understanding complex forms of mental functioning it is often necessary to clarify the structural and functional basis of brain machine. With relative precision he distinguishes the following three functional blocks: a block of maintaining the state of alertness and awareness, block the receipt, processing and storing exteroceptive information, programming block and control of voluntary activities based in the frontal lobes.

All three functional blocks are involved in the organization of any kind of complex mental activity and behavior. Having this perceived, the higher mental functions present

complex, hierarchically structured functional systems whose links are afferent and efferent type deployed in the distributed, remote cortical and subcortical regions of the brain. General properties of functional systems have a high degree of plasticity, mutual functional interactions and the possibility of their reorganization after the effects of pathological factors. Based on that, every psychic function relies on the joint work of different parts of the brain which are part of the given functional system. One and the same psychic function can be damaged at various locations of brain injury, but the quality of dysfunction will be specific for a particular area (Očić, 1988).

Neurobiological Basis of Executive Functions

The main feature of the prefrontal area is late maturing. In the prefrontal cortex three main functionally distinctive areas can be distinguished:

- Dorsolateral area - located on the outer side of the prefrontal cortex, the phylogenetically youngest part of the prefrontal cortex with rich reciprocal projections from the posterior association areas that integrate visual, auditory and somatotask data.
- Mediobasal area - focused on the anterior part of the cingulate cortex, which surrounds the corpus callosum. It has the richest links with structures that are considered relevant for memory, but also for other cognitive processes.
- Orbitofrontal area - has intense two-way connections with the amygdale, which enable a special role of "mapping" internal information such as motivation and emotion.

The frontal - subcortical circuitry should be mentioned - the loop of the frontal cortex with subcortical structures, such as anterior cingulate areas form a network for auto-regulative processes, initiative, and motivation, cognitive control of attention, concentration, and integration of complex information.

Functional Organization of the Frontal Lobes

Stuss and Benson (Stuss, 2002) have incorporated in their interpretation of functional organization of the frontal lobes the basic Luria settings, that is the concept of programming, control and regulation of behavior by emphasizing the function of self-awareness. This highest level of functioning of the frontal lobes, as well as an exclusive feature of the human species, is comparable to the concept of metacognition considering that self-consciousness includes introspective ability, and knowledge of their own knowledge. In the case of frontal lesions, it is the inability to use otherwise preserved knowledge about their own condition in order to regulate behavior, which manifests in the inability to self-observe, self-assess the problem, to grasp their consequences and make the appropriate decisions (Očić, 1988).

Model of cognitive control of Norman and Shallice's (Krstić, 2011), is based on the idea that there are two levels of behavior control: the lower level is used to start a number of

activities that arise without conscious intention, and parallel with other activities; a higher level of control behavior, for whose involvement is needed a conscious decision with adequate preparation and the execution itself is controlled by Supervisory Attentional System (SAS). According to this hierarchical model, the first level of cognitive architecture consists of specific cognitive units related to perception, language and practices. By their integration the behavioral patterns necessary for the control of routine activities arise on the other levels. The third level corresponds to the routine selection of mutually competitive cognitive schemes and functions by well-established rules in familiar situations. In new and unfamiliar situations which require suppression of habit, the activation of top-level cognitive control of SAS occurs. The model of supervisory attentional system includes a two-step control of executive motor and mental actions. This system renews its functions through modulation of activities in lower level on a flexible way by changing patterns of behavior. This highest level of cognitive control is in the responsibility of the prefrontal cortex, and the absence of its functions (in case of damage) is expressed through the rigid and perseveration behavior which is in the power of lower-level cognitive control (Očić, 1988).

Basic Executive Functions

Although today there is no absolute agreement on the separation of the most important components of executive functions, most often as with Luria (Luria, 1983), include aim selection, planning, inhibition of irrelevant responses and impulses, monitoring and regulating activities, as well as the evaluation of action outcomes. Voluntary attention control, working memory and temporal organization of behavior, also stand out as a special executive functions (Krstić, 2011). Working memory - such as by Badli stated (Baddeley, 1986) can be viewed as a multi-component system, whose function is to periodically store and manipulate information. This system, especially its control component is considered to be a prerequisite for the planning, selection and action control. Behavior disorder and patients cognitive impairments of executive functions is named Dysexecutive syndrome, and becomes regularly used in the scientific literature. Inhibition - is necessary for the smooth maintenance of a "mental set". In the base of behavioral inhibition three interrelated executive inhibitory processes exist: inhibition of the predominant responses; stopping initiated responses, control of interference. Temporal Coding - is very important executive control mechanism of information delay in short-term memory (Baddeley, 1986).

The Concept of Executive Functions

Even Lezak (Lezak, 2004) in her mid-career work indicated that the executive functions consist of skills that allow a person to successfully engage in independent, purposeful and self-contained behavior. According to the initial treatment she explains the role of executive functions in four categories:

- Formulation of intentions - is in accordance with the subject's needs and desires and designs their implementation in the future. This behavior is determined by the

concept of a purpose. It includes motivation, insight into their self-psychological and physical characteristics, as well as relation to the environment. Her clinical correlate makes apathy, difficulty in carrying out an activity without environmental stimuli and instructions, passivity and inertia.

- Planning - includes the ability to specify the strategy which will be implemented and the intentions of the aim with learning to distinguish between desires and real circumstances, anticipating through the consideration of alternatives and affiliation. Necessary conditions of effective planning are good impulse control, good capacity of maintenance attention and memory.
- Performing planned activities - includes its realization through self-initiation of integrated behavioral and physical activity, maintenance, stopping and changing individual behavior and movement sequences. Productivity and flexibility are the indicators of self-regulation movement and behavior. Clinical correlate makes a global slowing and the impoverishment of behavioral patterns and movements, perseverative, stereotypes and lack of adaptation.
- Efficiency of achievement - includes the ability of monitoring, self-correction and quality control aspects of behavior. Dysfunction of this component is assessed by analyzing the nature of errors, the patient's ability to grasp, his reactions to them, and behaviors that are used to overcome them (Očić, 1988).

Stussy and Benson (Stuss, 2002) almost identically defined executive functions, including the capacity to anticipate, set aim, plan, activate a number of attempts, and verify the results and comparison to the planned ones. The same authors report an interesting fact that one of the phylogenetically youngest brain structures - prefrontal cortex - is crucial for the highest human mental attributes, or the function of self-consciousness.

The Role of Executive Functions in Motor Activity and Walk

Up until recently, stroke was generally regarded as a mostly automatic motor task, which requires minimal cognitive engagement to a higher level. Growing evidence, however, link changes in executive function and attention aimed at healthy walk and walk with disabilities (hindered rotation). The relationship between executive function and attention and the walk itself becomes more important when monitoring patients with irregularities while walking. Multiple neuropsychological impacts on walking and interdependence of movement control and appropriate behavior are connected by cause and effect. It is manifested in the part of the individual awareness of the destination, the ability to adequately control the limbs which produce walk and mobility through the often complex environment in order to successfully get to the destination. Methods of visualization demonstrate, that is show the involvement of frontal activity during the movement. This review covers the importance and relevance of two specific cognitive function, executive function and attention in deriving walk during normal walking as well as in pathological conditions.

Executive functions refer to a number of cognitive processes that use and modify information from many cortical sensory systems in the anterior and posterior regions of the brain in order to change and produce both behavior and movement. These integrative

functions including motor and behavioral components necessary for effective targeted actions are the basis of the ability to perform activities of daily living.

The role of prefrontal areas in motor functioning refers to the ability to master the specific mechanisms that allow individuals a certain level of motor competence in the implementation of independent, purposeful goal-directed behavior. Since the frontal areas are composed of a number of subsystems, which are carriers of various functions and as these systems mature at different speed, their role in motor functioning can be viewed by following certain mechanisms of motor expression, which harmoniously compile motor functioning as a whole. The wealth of functions, its position and participation in the various components of human activity, indicate that their importance in motor performance is reflected through clearly differentiated, organized and properly executed motor actions.

Biomechanics of Human Movement and Characteristics of Effective Walking

The man's walk assumes an upright position and consists of a series of rhythmic body movements, which are accompanied by postural adjustment, that aims to move the body from one point to another in terms of the most efficient ways of using energy. Mostly simplified, the walk can be seen through the way of moving the gravity line that is set directly in front of the first sacral disk. The center of gravity is considered to be the most important factor in monitoring the course of walk, although it moves only a few inches vertically and laterally within the pelvis. These shifts are important in "absorbing" ground reaction force. In studying walking patterns, it is usual to start with walk cycle that begins when one foot makes contact with the ground and ends when the same foot again starts this contact. With this in regard, the cycle of walk is defined and analyzed. The walk cycle is a period between the ipsilateral (equilateral, isosceles) contacts on the feet and it is divided into two phases or two periods: a) phase support - which means the foot contact with the ground, b) phase pendulum - which means the absence of this contact.

Normal walking pattern or walk of a healthy individual (effective walk) is easily recognized because it represents a rhythmic cycle that repeats itself in a coordinated manner. The effectiveness of the walk is observed as followed: a) the stability in the support phase on both and on one leg; b) The efficient transfer of weight from one leg to another, running rhythmically and relatively fast setting foot during the swing phase of support and c) alternative transfer and movement of the body weight forward, which is happening the most during the swing phase. Related to the aforementioned, the walk is often defined as the movement of the center of gravity in space that requires the least energy consumption (Pavlović, 2006).

Kinematic Walk Analysis

Under the kinematic elements the scope and the direction of movement are implied, but not the force. In the frame of tracking the kinematic analysis, the walk is accompanied by a characteristic scheme and eventual deviations from normal. Starting the walking activity of the hip flexors, there is a mild abduction and external rotation of the hip joint until the moment of the heel support, when scheme changes quickly in the direction of adduction and

internal rotation which last during the time of load. Adduction here is the result of lowering the pelvis contrary to the support and the palm-phase pelvis in a neutral position, the hip is rotated inside and trunk begins to move in order to facilitate the transfer of weight.

Walk Parameters

Spatial walk parameters represent monitoring and measuring foot contact in the time area during the walk cycle: length of the pace is the distance at which one foot is placed in front of the other (normal 37.5 to 50 cm) and the length of the double pace ("Strida") is the distance between the two successive set of the same foot (normal range 75 - 100 cm). Shoes and height of a person have a direct impact on this segment.

Temporal walk parameters are related to the time in the walk cycle and include the duration of the support phase, the duration of the swing phase, during single support, double support time and time overall walk cycle. The combination of temporal and spatial parameters, allows the calculation of the speed of walking. Walking speed affect the kinematics.

The usual normal walking speed on ground level is 82 m/min. Adult males were 5% faster (90 m/min), women were 6% slower (77 m/min). The number of paces varies from 70 to 130 per minute. The ratio of time spent in different stages of the walk, also varies with speed. When walking faster, the length of paces increases and the increase in speed results in a proportional phase of extension and shortening of the phase of the oscillation of the support phase.

Locomotor Activity and Movement Control

In the walking control of the man the large number of components of the motor mostly motor cortex, basal ganglia, cerebellum, and parts of the reticular formation of the spinal cord are included. Determination of these structures was realized in many ways, primarily by registering increased electrical activity of their neurons during walking, and electrical stimulation of these regions leads to the appearance of movement similar to walk. By involving large number of structures of the CNS during walking witnesses the complexity of this seemingly simple motor action. This is clearly seen in the analysis of mechanisms of walking: alternating flexion and extension of the limbs; successively shifting the body weight from one foot to the other while maintaining impeccable balance and a certain body posture.

Cortex has multiple roles in the process of walking. Thus, motor cortex receives not only somaesthetic, but the vestibular and visual information. Based on this information forms the representation of space in the CNS, that is the reference axis related to the objects in the environment and in relation to the effects of gravity. This spatial pattern may be required for carrying out movements in the space for walking. Second, programs for sequences of voluntary movements involved in locomotion are encoded in the primary motor area, where it was sent by corticospinal tract in the spinal cord. Basal ganglia with motor cortex have a very important role in locomotion. They perform the integration of visual and proprioceptive information, then participate in the corresponding sequence of movements necessary to locomotion, and perform the initiation and cessation of walking.

Balance and Balance Disorders

Balance is the ability to keep the body above the surface of the support in a variety of anti-gravity conditions. This is coordinated muscle groups activity that opposes gravitational forces, which is a prerequisite for the development of good posture, ability to maintain attitudes, performance and movement skills acquisition. Balance is not static, it means continuous alignment position whether to make a barely perceptible movement or to make a change in tone. Balance basically involves an integrated response development of labyrinth and optical erection, development of balance reactions and their integration with proprioceptive and tactile sensations. Good balance and good posture mean that the postural reflexes well developed and automated.

The loss of one sensor is a kind of serious disorders, although information from other areas of well-compensated for these deficits. Balance depends on many factors: the mature central nervous system, of pathological changes in the CNS, the mobility of joints, mobility of the spinal column, the mobility of the limbs, muscle strength, the state of the sensory organs, the presence of pain, the surface of support, the weight, height, etc. The disorder was any of these factors in any form may lead to disturbances of balance.

Motor Functions and Walk in Patients After a Stroke

In typical circumstances, walk is the automatic activity in which you do not pay attention to the sequence of execution. You start walking activity voluntary, and centers in the central nervous system, take over the role further on, until the execution of the activity. In situations of the damaged central nervous system, such as after a stroke, the scheme or the majority of them are no longer present. Then the patient has to adapt to new circumstances and benefit schemes available to him/her. Kinematic and kinetic aspects of locomotion in these patients vary depending on the severity of the damage, the degree of recovery and the use of various compensatory mechanisms. Classically recognized is the scheme of "circumduction while walking." The energy consumption of these patients compared to the normal walking is significantly higher. The dynamics of the recovery of the walk and function of the lower extremities is done through various stages hierarchically arranged according to the conquest of certain activities of the movement (Pavlović, 2006). By following the course of recovery one must bear in mind that each patient has "their plateau" of recovery.

The study conducted on patients after a stroke in the rehabilitation process show the usual kinematic characteristics of walking. Walk as a significant functional activity in a patient after a stroke, is a process which is carried out in phases. It starts with a phase of practicing motor control with an emphasis on symmetry in all positions. It is believed that the neurophysiologic preparation for relatively satisfactory scheme is done when they gain control over some of the major muscle groups of stroke. At the time when motor activity is dominated by the basic pathological synergy, in a post-stroke patient, individual compensations related to weight of damage could be expected, which essentially contributes to the complication of abnormal patterns. Therefore, when it comes to practicing motor responses of the lower extremity necessary for walking, with the emergence of voluntary movements, one should go on practicing a combination of the so-called functional movement patterns required for walking (the elements necessary to support phase and phase swaying).

When conditions are right, the practicing walking in all directions is followed. In direct coaching, a slow pace is first used for the purpose of practicing and emphasizing each phase of walking (Pavlovic, 2006).

From the standpoint of biomechanics, synergies are considered to be co-ordinated actions of joints and muscles in order to achieve the set goal. Within the clinical neurorehabilitation, motor synergies are usually defined as stereotyped movements of the whole limb which suggest the loss of independent joint control and limited ability to coordinate. The walking schemes thus become less adaptable, making it difficult for the performance of a large number of functional motor tasks. In the framework of neurorehabilitation synergies are considered side effects, and treatment is aimed at "breaking synergies". These synergies are called obligatory, and the most basic division is on extension and flexion synergy.

Early walk, which means the neutral walk, which is not yet won stability and controlled mobility can be a motivational mechanism for activation of the patient, but it entails great risk of unwanted and often unexpected deviation in a given activity. Therefore, it is considered that a functional walk should practice, once in a standing position one can gain satisfactory level of balance, and controlled mobility (Pavlović, 2006).

Problem and Objective

The aim of this study was to investigate the correlation between ability to organize activity and attention, and walk disorder in patients after a stroke, and how that relationship affects the achievement of functional independence of patients.

The ability of planning and organization of activities as a cognitive trait that allows precise strategies that will be implemented and the intentions of the aim, represents the highest level of functioning of the frontal lobes. The ability of planning and organization activities is a privilege of a human and is comparable to the metacognition that is, with awareness of their own knowledge. It includes all the components from which the "goal-directed behavior" derives and that is formulation of intention, action planning, carrying out planned activities and verification of actions performed. The ability to organize activities is based on a cognitive process which use the modify information from many cortical and sensory systems in the anterior and posterior regions of the brain, and thus to control, modify, and produce activity. It is linked to the functioning of the frontal lobe of the brain and the corresponding network. The area of the prefrontal lobe is associated with cognitive aspect of the ability to organize activity (EF), the anterior parts are included in the aspect of the behavior of self-regulation (inhibition and self-awareness), while the back parts are included in the process of concluding. Neuroimaging studies that attempt to determine the topographic frontal lobe activity, show inconsistent results (Collette et al., 2008) and suggest that different tasks intended for the functions of the frontal lobes activate not only different frontal areas, but other areas of the brain.

Cognitive impairment after a stroke may be complex, particularly in the involvement of the cerebral hemisphere or the frontal lobes, because depending on the lesion, executive function will be more or less effective. One investigative study suggests a significant association of the left frontal lobe damage and particular characteristics of executive functions (for example - the ability to plan), while damage to the right frontal lobe are of great importance to the quality of executive functions. This study highlights the importance of

multicomponent approach to the assessment and rehabilitation of patients with stroke (Jodzio, Biechowska, 2010). Function of walking is from all motor functions commonly affected. The walk of healthy people provides the basis for the assessment of pathological walk and ways of resolving the dysfunctions. In the control of human walking a large number of components of the motor system, primarily motor cortex, basal ganglia, cerebellum, parts of the reticular formation and spinal cord is included. Cortex plays an important role in the process of walking, it is provided not only by somaesthetic but vestibular and visual information as well. Based on this information the representation of space in relation to objects in the environment and in relation to the effect of gravity is formed, this spatial pattern is necessary for the execution of movement in space and walking. The programs for voluntary movement sequences involved in locomotion are encoded in the primary motor area. By identifying the role of specific structures of the CNS in function walk it can be reached in several ways: by registering increased electrical activity of their neurons during walking, by electrical stimulation of specific areas that cause similar movement speed and specific test techniques and procedures that can be linked cognitive and motor function.

Anticipating the possibilities and limitations in patients after stroke, the aim of this study was to determine the relation between the ability to organize activity and attention to irregularities while walking. Determination of cognitive competence (or dysfunctions) and the dysfunctions walk in patients after a stroke, is within the scope of the theoretical part of this study. Practical significance of the research will have full confirmation if the established connection is important for the application of medical rehabilitation and enable the achievement of a high degree of functional independence of patients after stroke. Since the achievement of functional independence of one of the leading and final goal of medical rehabilitation of patients after stroke, and that the presence of cognitive deficits may affect the course and outcome of the end of the process, the scientific justification for this research is the multidisciplinary approach to the diagnosis, and in treating these patients.

RESEARCH METHODOLOGY

Basic methodological principles of research is based on the comparison of results between the two groups of respondents, which consisted of the evaluated patients after a stroke and patients without neurological damage, in order to identify possible differences between individual variables of the research. Kao as a target study the differences within the group surveyed variables in post-stroke patients with cognitive impairment and without cognitive impairment will be analyzed.

Research Instruments:

Wisconsin Card Sorting Test - WCST

The main purpose of this test is to detect perseverativity and mental rigidity of the surveyed companies. The ability to plan and organize activities as cognitive trait that allows precise strategies by which will be implemented the intentions and the accomplished aim represent the highest level of functioning of the frontal lobes. Desires or will, planning,

anticipation, intent implementation and verification of the performed actions were assessed by WCST - Wisconsin card sorting test (Heaton, 1981). It is card sorting test and the most popular test for detecting perseverativity and mental rigidity. It was primarily for assessing abstraction in healthy respondents.

Trail Making Test - TMT A/B

This is a test of the neuropsychological test battery box and is used for evaluation of the flexibility of attention. The attention is the direction and focus of mental activity to something specific, whereby the orientation is determined by selectivity and duration, and focus by the possibility to ignore distraction. Attention has been tested using the TMT A / B test. It has been recognized since its inception as a technique sensitive to the effects of brain damage in general (Reitan, 1958).

Mini Mental State Examination - MMSE

Mini Mental State Examination - MMSE is a test for evaluating the cognitive state of patients, screening option, easy-to-use, sensitive and gives valid results. Source: Folstein MF, Folstein SE, McHugh PR., (1975): "Mini-mental state: a practical method for grading the cognitive state of patients at the clinic," J Psych.

Functional Ambulation Category - FAC

The test is intended to assess the quality of movement and performance of motor tasks in patients after stroke. Functional Ambulation Category - FAC test or functional test movement (Holden et al., 1984) is a simple measuring instrument that includes assessment of walking ability in hemiplegic patients. The test has six terms.

Step Test

Step test (Hill et al., 1990) is primarily used to assess the ability to perform motor task in overcoming certain obstacles including spatio-temporal parameters and balance. In this test evaluates the index of the feet of the motor (Motoricity leg index).

Functional Independence Measure - FIM

The main purpose of the test is to assess the functional independence of patients with neurological impairments. The achievement of functional independence is leading and final goal of medical rehabilitation. FIM test with a minimum of data provides an adequate, prompt, valid, general assessment of functional capacity of patients with neurological impairments and as such is accepted in the world. FIM test covers six areas, and within these areas the eighteen tasks.

RESEARCH RESULTS

General Characteristics of the Sample Survey Results

The sample after a stroke consisted of 50 subjects, female = 24 N (48%) and males n = 26 (52%). These were the patients that were included in the process of rehabilitation after a

stroke. The range of their age was from 54 to 80 years, mean value (hereinafter referred to as AM) was 69.99 years, the standard deviation (hereinafter referred to as SD) was 7.71 years . 22 patients (44%) who had a right-hand hemiparesis and 28 (56%) dextral hemiparesis.

The sample of individuals without neurological damage also consisted of the 50 respondents, females N = 27 (54%) and males N = 23 (46%), selected randomly, in the appropriate age. Their age range was from 51 to 82 years, M = 67.18 years, SD = 9.27 years. This group of people consisted of persons who did not have in their history any neurological findings of symptoms of acute and chronic neurological diseases, Parkinson's disease, multiple sclerosis, dementia and depression.

Statistical tests that were applied for assessing differences between the groups have shown that the group of persons after a stroke and the groups of individuals without neurological damage did not differ significantly in terms of gender, age and educational structure, they differ.

Significantly in terms of marital status, among persons after stroke compared to a group of people without neurological damage more than one person was in a marriage, a small number of divorced and those who are unmarried / single. Risk factors for hypertension and type II diabetes was significantly more common in the group of persons after the stroke than in the group of individuals without neurological damage (Table 1).

Table 1. Shows structure of groups of patients in terms of demographic characteristics and risk factors and test results for testing the statistical significance of differences between groups in terms of these characteristics

Characteristic		Persons after a stroke	Persons without neurological damage	The value of statistics for testing the difference	Stat. Signif.
<i>Gender</i>					
	Male	26 (52%)	27 (54%)	$\chi^2 (1, N = 100) = .36$	p = .55
	Female	24 (48%)	23 (46%)		
<i>Age</i>					
	AM (SD)	69.99 (7.71)	67.18 (9.27)	t (98) = 1.58	p = .12
	Range	54 – 80	51 – 82		
<i>Education</i>					
	Four grades	6 (12)	10 (20)	$\chi^2(3,N=100) = 5,72$	p = .13
	Elementary	21 (42)	18 (36)		
	High	20 (40)	13 (26)		
	College / Higher	3 (6)	9 (18)		
<i>Marital satus</i>					
	Married	32 (64)	13 (26)	$\chi^2(3,N=100) = 20.87$	p < .001
	Widow/er	15 (30)	18 (36)		
	Divorced	3 (6)	11 (22)		
	Unmarried / Single	0 (0)	8 (16)		
<i>Risk factors</i>					
	Hypertension	25 (50%)	11 (22%)	$\chi^2 (1, N = 100) = 8.51$	p< .05
	Type II Diabetes	18 (36%)	3 (6%)	$\chi^2(1,N=100) = 13.562$	p< .001

Legend: AM - arithmetical mean, SD - standard deviation.

Table 2. Shows results of the tested flexibility of attention and visuomotor tracking by applying TMT A/B test to a group of patients after a stroke, and for a group of subjects without neurological damage

Parameter	Normative values of the test (seconds)				Persons after a stroke (seconds)				People without neurological damage (seconds)			
	AS	SD	min	max	AS	SD	Min	max	AS	SD	Min	max
TMT A	34.8	11.4	20	80	121.21	46.55	41	205	81.82	32.13	31	180
TMT B	80.9	28.8	35	165	310.75	72.82	110	350	201.98	84.71	30	300
					The difference compared to normative group							
TMT A					t(72) = -12.38, p<.001				t(72) = -9.21, p<.001			
TMT B					t(72) = -19.38, p<.001				t(72) = -9.04, p<.001			

Table 3. Shows statistical significance of differences in results of the tested flexibility of attention and visuomotor tracking application TMT A / B test between a group of persons after a stroke and a group of persons without neurological damage

Parameter	Persons after a stroke	Persons without neurological damage	U	r
	Middle range	Middle range		
TMT A	61.93	37.57	603.50 ***	.42
TMT B	67.66	32.07	328.50 ***	.64

Legend: U - value of the Man-Whitney (Mann-Whitney) in statistics, r - effect size.

People after a stroke from the sample have significantly statistically higher average value of the parameter TMT A from normative value, $t(72) = -12.38$, $P < .001$, and significantly higher values of the parameter TMT B from normative value, $t(72) = -19.38$, $p < .001$. In individuals without a neurological damage of TMT is determined by the value of A $t(72) = -9.21$, $p < .001$, and the TMT B, $t(72) = -9.04$, $p < .001$.

Table 4. Shows results of tested planning skills and perseverativity by applying WCST test for a group of patients after a stroke and a group of subjects without neurological damage

Parameter	Normative values of the test		Persons after a stroke				Persons without neurological damage			
	AS	SD	AS	SD	min	max	AS	SD	min	max
Categ.	5.4	1.3	1.28	1.39	0	5	2.53	2.03	0	8
Pers. er.	12	10	7.92	4.81	0	22	3.16	3.25	0	12
	Differences in relation to the normative group									
Categ.	t(73) = 12,36, p<.001 t(73) = 10,49, p<.001									
Pers.er.	t(73) = 2,73, p<.01 t(73) = 5,69, p<.001									

Legend: Categ. - Number of categories; Pers. er. - Number perseverative errors.

The results of the Mann-Whitney tests show that a group of people after a stroke had in average higher value of the parameter TMT A in relation to the group of persons with no

neurological damage, $U = 603.50$, $p < .001$, and the effect size was moderate ($r = .42$). Also, persons after a stroke in average had higher values of the parameter B TMT from persons without neurological damage, $U = 328.50$, $p < .001$, and the effect size was large ($r = .64$).

The results of the Mann-Whitney tests showed that the group of patients with post-stroke hemiparesis of the right hand did not differ significantly in terms of the parameter TMT B (mean range = 23:47) from subjects with hemiparesis of the left side (mean range = 20:94), $U = 523.50$, $p = 0.40$.

The results of t-test showed that persons after a stroke in our sample had significantly lower number of categories on the WCST test compared to the normative sample, $t(73) = 12.36$, $p < .001$. Perseverative errors were statistically significantly lower among persons after a stroke compared to the sample values obtained by the sample normative test, $t(73) = 2.73$, $p < .01$.

Persons without neurological damage from the sample had an average of a statistically significant number of categories in WCST test compared to the test norms, $t(73) = 10.49$, $p < .001$. Also, they had a statistically significantly lower number of perseverative errors compared to the test norms, $t(73) = 5.69$, $p < .001$.

The results of the Mann-Whitney tests show that a group of people after a stroke in average achieved fewer categories (MDN = 1) than people without neurological damage (mdn = 2), $U = 729.50$, $p < .001$, effect size was moderate ($r = .35$). Also, the persons after a stroke in the average had more perseverative errors (mdn = 8) compared to the persons without neurological damage (mdn = 2:50), $U = 509.50$, $p < .001$, the effect of group membership of the difference is large ($r = .51$).

Results Mann-Whitney tests showed that subjects with post-stroke hemiparesis of the right hand achieved significantly lower (.05 significance level) the number of categories (mean rank = 18:16) on the WCST than respondents with hemiparesis of the left side (mean rank = 26.54), $U = 155.00$, $p < .05$.

Table 5. Shows statistical significance of differences in the results of the test planning skills and perseverativity by applying the WCST test between groups of persons after a stroke and a group of persons without neurological damage

Parameter	Persons after a stroke		Persons without neurological damage		U	r
	Middle range	mdn	Middle range	mdn		
Categ.	40.09	1	60.11	2	729.50 ***	.35
Pers. er.	65.31	8	35.69	2.50	509.50 ***	.51

Legend: Categ. - Number of categories; Pers. gr. - Number of perseverative errors; MDN - median, U - value of the Mann-Whitney in statistics, r - effect size; *** $p < .001$.

Table 6. Shows that among the persons after a stroke, 22% of them categorized as with no cognitive impairment according to the MMSE test results, 72% in the category of mild cognitive impairment, and in 6% of test results indicating severe cognitive impairment.

Among subjects without neurological damage, no person were found with mild and severe cognitive impairment according to the results of the MMSE test.

Table 6. Tabulation of the level of cognitive functioning of examinees after stroke and the examinees without neurological damages of MMSE test

Total of points	Damage	Persons after stroke N(%)	Persons without neurological damage N(%)
24-30	Without cognitive damage	11(22)	50(100)
18-23	Light cognitive damage	36(72)	0(0)
0-17	Severe cognitive damage	3(6)	0(0)
Total		50 (100)	50 (100)
$\chi^2 (2, N = 100) = 63.93, p < .001.$			

The Chi-squared test showed that the difference between the two groups in terms of belonging to certain categories of cognitive impairment (non impairment) is statistically significant at 0.001, $\chi^2 (2, N = 100) = 5.15, p < .001$.

The results of Mann-Whitney test showed that there were no statistically significant differences in the level 0 .001 between the two groups of respondents in terms of average scores on FAC test, $U = 302.00, p < .001$, effect size was large ($r = .69$). The patients without neurologic defects have an average score of FAC (mdn = 5) compared to the post-stroke patients (mdn = 2).

Table 7. Shows the results obtained by evaluation of walk in patients after a stroke and patients without neurological damage by applying a functional test of movement

FAC score	Persons after a stroke		Persons without neurological damage	
	f	%	f	%
0 not walking independently	0	0	0	0
1 assistance of one person	16	32	0	0
2 verbal support	12	24	0	0
3 independent on the flat ground	5	10	0	0
4 obstacle assistance	9	18	12	24
5 completely independent	8	16	38	76
Total	50	100	50	100
Mdn	2		5	

Legend: mdn– median.

Table 8. shows statistical significance of differences in the results obtained by the evaluation of walk function among the surveyed people after stroke and surveyed individuals without neurological damage

Group	Middle range FAC scores	U	r
Persons after a stroke	31.54	302.00 ***	.69
Persons without neurological damage	69.46		

Legends:*** $p < .001$.

Table 9. Shows the results of measured walk frequency in patients after a stroke and patients without neurological damage

Walk frequency (number of steps per minute)										
Norm	Persons after a stroke					Persons without neurological damage				
	min	max	MV	SD	mdn	min	max	MV	SD	mdn
70-130	23.67	92.90	49.87	14.92	45.71	37.80	87.27	54.05	10.88	52.16

Legend: min - minimal determined value, max - maximal determined value, MV - mean, SD - standard deviation; MDN - median.

Table 10. Shows statistical significance of differences in the results obtained by measuring the frequency of walk among persons after a stroke and those without neurological damage

Group	Walk frequency (number of steps per minute) Middle range	U	r
Persons after a stroke	42.53	851.50 **	.
Persons without neurological damage	58.47	27	

Legend: ** p <.01.

Table 11. Shows the results of measured walking speed compared to normative values in patients after a stroke and patients without neurological damage, and the statistical significance of differences in walking speed between persons after stroke and those without neurological damage

Grou		Walking speed (m/min)						U	r
		MV	SD	mdn	min	max	Midle range		
Norms	Men	86	/	/	/	/			
	Women	77	/	/	/	/			
Persons after a stoke	Men	10.48	5.06	8.11	5.45	20.00	25.74	12.00 ***	.85
	Women	7.59	1.61	7.28	5.50	10.71			
Persons without neurologila damage	Men	34.03	8.57	31.58	19.35	54.55	75.26		
	Women	26.59	15.79	66.67					

Legend: min - minimal determined value, max - maximal determined value, MV - mean, SD - standard deviation; MDN - median, *** p <.001.

According to the Mann-Whitney test (Table 10), persons with no neurological damage had in average, a higher frequency of walk (mdn = 45.71) compared to persons after a stroke (mdn = 52.16) and the difference is statistically significant at the 0.01 level, U = 851.50, p <.01, and the magnitude of the effect is small (r = 0 .27).

Results Mann-Whitney tests showed no statistically significant differences in the frequency of walking between subjects with right-hand hemiparesis (mean range = 24.13) and hemiparesis of the left side (mean range = 22:17), U = 225.50, p = . 62.

Table 12. Shows the results of measured length of steps in patients after a stroke and patients without neurological damage and statistical significance examinations of differences in the length of stepping between the groups

Step length (cm)						
	min	max	MV	SD	mdn	Middle range
Persons after a stroke	14.00	35.00	23.28	5.39	23.00	25.84
Persons without neurological damages	26	75	52.28	8.29	52.00	75.16

Legend: min - minimal determined value, max - the highest determined value, MV - mean, SD - standard deviation; MDN - median, *** $p < .001$.

According to the Mann-Whitney test (Table 11), persons without neurological damage in average, had a higher walking speed (mean rank = 75.26) than persons after a stroke (mdn = 25.74) and the difference is statistically significant at 0.001, $U = 12.00$ $p < .001$, and the effect of group membership on the walking speed was high ($r = 0.85$).

The results of the Mann-Whitney tests showed that the group of patients with post-stroke (hemiparesis lat. Dextre) right-hand hemiparesis did not differ significantly in terms of walking speed (mean rank = 25.08) than subjects with (hemiparesis lat. sinistri) left hemiparesis (mean range = 21:48), $U = 207.50$, $p = 0.36$.

The Mann-Whitney tests results showed that patients without neurological damage, in average, had significantly higher assessment of foot dorsiflexion in patients after a stroke, $U = 48.50$, $p < .001$, and the effect of group affiliation on the result was large ($r = .94$) (Table 13).

Also, the results of Mann-Whitney test (Table 13) showed that the patients without neurologic defects had a higher score in average of knee extension in post-stroke patients, $U = 221.00$, $p < .001$, and the effect of group affiliation on the result was large ($r = .81$).

Results Mann-Whitney test (Table 13) showed that patients without neurological damage, in average, had significantly higher hip flexion in patients after a stroke, $U = 375.00$, $p < .001$, and the effect of group affiliation on the result was large ($r = .70$).

Table 13. Shows the results of the Motor Index Leg test in patients after a stroke and those without neurological damage and the results of the statistical significance of differences between groups

Parameter	Persons after a stroke			Persons without neurological damage			U	R
	f	%	Middle range	f	%	Middle range		
Dorsiflexion of foot			26.47			74.53	48.50 ***	.94
No movement	3	6		0	0			
In contrast to gravitation	46	92		1	2			
Normal	1	2		49	98			
Total	50	100		50	100			

Parameter	Persons after a stroke			Persons without neurological damage			U	R
<i>Knee extension</i>			29.92			71.08	221.00 ***	.81
No movements	2	4		0	0			
In contrast to gravitation	43	86		4	8			
Normal	5	10		46	92			
<i>Total</i>	<i>50</i>	<i>100</i>		<i>50</i>	<i>100</i>			
<i>Hip flexion</i>			33.00			68.00	375.00 ***	.70
No movements	0	0		0	0			
In contrast to gravitation	45	90		10	20			
Normal	5	10		40	80			
<i>Total</i>	<i>50</i>	<i>100</i>		<i>50</i>	<i>100</i>			

Legend: *** $p < .001$.

Table 14. Tabulation of the results obtained by evaluation of maintaining a balance by using the STEP test in patients after stroke and those without neurological damage

Balance	Persons after stroke		Persons without neurological disorder	
	f	%	f	%
Have	29	58	49	98
Partially	4	8	1	2
Don't have	17	34	0	0
<i>Total</i>	<i>50</i>	<i>100</i>	<i>50</i>	<i>100</i>

$\chi^2 (2, N = 100) = 23.93, p < .001$

Table 15. Spreadsheet of results given by prediction analysis of movement characteristics based on indicator of planned behavior ability

Variable	Coef	Rc	Rc ² (%)
	.	.	
Gait frequency	.142	.416	17.31
Gait speed	.698	.574	32.95
Stride length	.474	.611	37.33
Movement independence (FAC)	.269	.413	17.06
Foot flexion	.473	.417	17.39
Knee extension	.158	.777	60.37
Hip flexion	1.289	.823	67.73
Rc ²			54.1
Number of categories WCST	.780	.987	97.42
Number of perseveration errors WCST	.262	.878	77.09

Legend: FAC- functional movement test; WCST – Wisconsin Card Sorting Test; Coef-standardized coefficient of canonic function; Rc – structural coefficient; Rc² - squared structural coefficient
 Remark: Structural coefficients above .450 are marked.

Table 16. Balance maintenance spreadsheet evaluated from STEP test: Within respondents group after stroke considering attention flexibility result evaluated from TMT B test

Balance	TMT B> AS(N = 37)		TMT B< or = AS(N = 13)	
	f	%	f	%
Have	21	56.76	10	76.92
Partially	3	8.11	0	0
Don't have	13	35.13	3	23.08
Total	37	100.00	13	100
$\chi^2 (2, N = 50) = 1.90, p = .270$				

Legend: TMT B > AS – In the TMT B group of persons of which the result is greater than the mean of the sample determined after one stroke; TMT B < or = AS – TMT B group of persons who have a score below the mean established for people after a stroke or equal to it.

Table 17. Tabulation of results of measured gait speed, gait frequency and stride length for patients group after stroke, given the results of the estimated value of the flexibility of attention TMT B test

Parameter	TMT B> AS(N = 37)			TMT B< or = AS(N = 13)		
	AS	SD	mdn	AS	SD	mdn
Gait speed (m/min)	78.44	21.59	82.50	65.83	26.52	74.00
Gait frequency (number of steps in min.)	48.29	14.18	44.50	54.69	17.60	49.61
Stride length (cm)	22.50	5.17	21.50	25.58	5.88	26.50

Legend: TMT B > AS – In the TMT B group of persons of which the result is greater than the mean of the sample determined after one stroke; TMT B < or = AS – TMT B group of persons who have a score below the mean established for people after a stroke or equal to it.

Table 18. Tabulation of results for testing the statistical significance of differences in gait speed, stroke frequency and stride length between patients after stroke, given the results of the estimated value of the flexibility of attention using TMT B

Parameter	TMT B> AS (N = 37)	TMT B< or = AS (N = 13)	U	R
	Middle ranking	Middle ranking		
Gait speed (m/min)	26.03	19.92	161.00	.18
Gait frequency (number of steps in min.)	23.10	28.71	165.50	.17
Stride length (cm)	22.67	30.00	150.00	.22

Legend: TMT B>AS – In the TMT B group of persons of which the result is greater than the mean of the sample determined after one stroke; TMT B < or = AS – TMT B group of persons who have a score below the mean established for people after a stroke or equal to it; U – Mann-Whitney U statistic, r – effect size; * p< .05.

Table 19. Tabulation of the results of Man - Whitney test for differences in gait parameters between persons with cognitive impairment and no cognitive impairment in a group of subjects with stroke

Parameter	Middle ranking reviews		U	r	p
	People after stroke without cognitive impairment	People after stroke with cognitive impairment			
	n = 11	n = 39			
Gait speed (m/min)	34.50	22.96	115.50*	.23	.210
Gait frequency (number of steps in min.)	33.91	23.13	122.00*	.21	.87
Stride length (cm)	36.77	22.32	90.50*	.29	.004

The difference between two groups of patients, regarding the affiliation to balance maintenance categories by STEP test, is not statistically important according to Mann-Whitney test results, χ^2 (2, N = 50) = 1.90, p = .270.

The Mann-Whitney test results showed that there was no statistically significant difference in gait speed, U = 161.00, p = .190, in stroke frequency, U = 165.50, p = .229, and the stride length, U = 150.00, p = .115, between persons after stroke, which differ in respect to TMT B results. Accordingly, the results do not suggest in favor to our staged third hypothesis.

There was a statistically significant difference in stride length between patients after stroke with and without cognitive impairment ($p < .05$). Persons without cognitive impairment, on average, have significantly longer step from persons with cognitive impairment. There is also a difference in the gait speed and frequency between persons with and without cognitive impairment after stroke. Persons without cognitive impairment have faster gait and larger gait frequency, statistical value ($p < .05$).

People with a large number of categories and a smaller number of perseveration errors on the WCST test, have a higher degree of functional independence in self-care and socialization.

Table 20. Tabulation of the results of the prediction to achieve functional independence in self-care and socialization based on indicator of planned behavior of patients after stroke

Variable	Coef.	Rc	Rc ² (%)
Self-care	.133	.565	31.9
Socialization	.931	.993	98.60
Rc ²			37.6
Number of categories WCST	.799	.989	97.8
Number of perseveration errors WCST	-.241	-.872	76.0

Legend: WCST – Wisconsin Card Sorting Test; Coef-standardized coefficient of canonic function; Rc – structural coefficient; Rc² - squared structural coefficient.

Man-Whitney test results showed a statistically significant difference in the following subscales of FIM test between persons after stroke with and without cognitive impairment:

self-care, sphincter control and locomotion. It was also found that patients without cognitive impairment achieved on average significantly higher scores on the subscales of communication and socialization than patients with cognitive impairment, the differences were statistically significant at $p < .05$.

Table 21. Tabulation of statistical significance of differences in the results of all investigated areas using the FIM test in the group of patients after stroke with and without cognitive impairment

Subscale	Persons after stroke without cognitive impairment	Persons after stroke with cognitive impairment	U	r
	Средњи ранг	Средњи ранг		
<i>Area</i>				
Self-care	33.64	23.21	125.00 *	.21
Sphincter control	29.68	24.32	168.50 *	.11
Locomotion	33.00	23.38	132.00 *	.19
Comunication	38.36	21.87	73.00 *	.33
Socialization	38.73	21.77	69.00 *	.34

Legend: U – Mann-Whitney U statistic; r- effect size; * $p < .05$.

DISCUSSION

Cognitive deficits such as attention deficit disorder, reduced efficiency of control functions, memory disorders and reduction of sensory-perceptual capabilities are common lasting effects of stroke. By using neuropsychological tests it can be identified even the most subtle cognitive impairment. Thus one can get a neuropsychological profile of patients after a stroke, with its strengths and weaknesses, and so we have a baseline of his mental competence, which may be of importance for inclusion in the medical rehabilitation. Whether and in what respect are assessed cognitive skills, motor skills and functional independence of patients after stroke and patients without neurological damage, we will follow the discussion through the results of our research.

Taking into account the functional organization of the frontal lobes responsible for the ability of organizing activity, often puts emphasis on the function of self-awareness. The highest level of functioning of the frontal lobes is comparable with metacognition, or awareness of one's own knowledge and ways of using it. In the case of frontal lesions it may be discussed the inability to use preserved knowledge about their own condition in order to control behavior, which may be manifested in impossibility of self-assessment and their own problems, to grasp their consequences and making appropriate decisions (Ocić, 1988). This fact is of great importance in the process of enrolling patients in medical rehabilitation as well as for the course of rehabilitation, until achieving the elementary level of functional capability. More research has studied the relationship between executive functions (EF) and the ability to walk.

In InChanti study (Seligmann et al., 2008), which treated 900 nondemented elderly (mean age 74.6 years and cognitive performance MMSE 25.5), visual-motor time-restricted test with dimensions of cognitive flexibility, the authors came to the conclusion that the low efficiency of executive functions was correlated with a decrease in walking speed on the track with obstacles. Similar findings were obtained by (Holtzer et al., 2006), they also talk about the connection between cognitive aspects and walking speed. These authors suggest that the pace of the elderly persons is complex task that requires a higher executive control of data processing and storage.

Another study (Hausdorff et al., 2005), on the basis of these results, we can conclude that more accurate walking with greater speed were correlated with the performance of other complex motor tasks. Also, the results of these InChanti studies suggest that association of executive functions to the walking increases as the complexity of the task increases. Most authors agree that the reduced efficiency of executive functions may participate in reducing the ability to move, but reason is not defined. Neurological patients have particular difficulty when executing another task while walking. Cognitive and motor tasks simultaneously given, question the ability of the locomotional system to perform a function of walking (Seligmann et al., 2008). Many studies show that there is a connection between executive function and functional recovery after stroke in patients who have been tested and have been involved in the rehabilitation process, but the results did not show that functional recovery of patients can be predicted based on the quality of executive functions (Nyhus, Barcelo, 2009). The results of this study are comparable with the above results of previous research while complementing them with new facts emerged from the results. A small number of categories achieved in favor of a reduced ability to specify a strategy that will perform a certain activity, and therefore difficult will be the ability of self-correction, as well as changes in the behavior of certain sequences that do not produce results. Although not covered by statistical analysis, it is worth noting that most of patients after stroke has quit the ongoing set. The reasons may be manifold, the most frequently mentioned as attention deficit, lack of concentration and motivation for cooperation. In patients after stroke, among others, number of attempts to achieve the first category was recorded, which just shows that they need more time to discover the actual principle of solving the task, or an appropriate strategy for carrying out certain actions (Table 4). Similarly, a small number of achieved categories is correlated with a large number of attempts to achieve the first category, as well as frequently giving up the current set, and a large number of perseverative response (dysexecutive syndrome characteristics). The results of this research the assessed executive functions in patients after stroke are consistent with the description of behavioral disorder caused by damage to the frontal lobes defined by Lezak 's (Lezak. 2004), describing them as difficulties in initiating actions, the decline of spontaneity, loss of initiative, perseveration and rigidity, difficulty in altering mental set, problems stopping certain sequences of complex activities, with symptoms of disinhibition and impulsivity and the inability to understand their own role in the social situation (Lezak, 2004). Qualitative description, recorded during the evaluation of the test subjects after stroke in our study, fully corresponds to the above mentioned facts that qualify the status of dysfunction in executive functions. Negative response to the feedback that requires retrial, destabilize patients and usually results in a waiver of further participation in the assessment. There is a significant latency between each response and the need for constant encouragement from the outside. If we take into account the results of a large number of previous studies, we find that the leading feature cognitive deficits in

cerebrovascular diseases, is in fact the presence of defects in executive functions (Galluzzi et al., 2005; Garett et al., 2004; Saschdev et al., 2004). The results of our study show that patients after stroke manifest problem with capacity planning and organizing activities, exhibit low efficiency in defining strategies that will be implemented intentions and achieved the objective. This is particularly important with patients after stroke, when they are in a position to independently perform certain structured, purposeful and goal-directed motor activity. What makes the problem more complex is inefficient anticipating the consideration of alternatives and affiliation, this is true for mental or motor activity and reduced efficiency of self-correction (Table 4). The results of this research related to the assessment of attention, show a significant statistical difference in achievement between the surveyed patients after stroke and surveyed individuals without neurological damage. These differences are related to the selectivity of the visual efficiency as well as visuomotor and visuoconceptual monitoring, with the monitoring of the special significance of the comparative two different conceptual sequence (Table 2). It is important to note that the efficiency of the achievements in this assess attention test (TMT A/B) affects the intellectual and educational level of the respondents, but the point is that it is very sensitive to brain damage. The results show that the tested patients after stroke had low achievement in the second part of the test that required a parallel monitoring of two different conceptual series and that in the same group of respondents is a significant difference in achievement between the first and second part of the test, which also has clinical significance. Surveyed patients after stroke, registered a large number of errors, as mistakes are punished by the total time spent, and it's one of the reasons for low efficiency. The qualitative performance of subjects with impaired frontal cortex, will characterize the harsh "shifting" of attention from one stimulus to another category (Krstic, 2011). The results of this study fit into this statement. With surveyed patients after stroke the quality of care, both in the field of tenacity, as well as in the field of vigilance disorders is manifested as significantly reduced, and therefore, the impact of distractors was undoubted and more important, distractors could be different stimuli, without any possibility of selection. Considering the very administration of a given test, which involves perceptive activity, surveyed patients after stroke with right-sided hemiparesis were reasonably less effective than the surveyed patients after stroke to perform left hemiparesis.

In left-sided hemiparesis, right hemisphere dysfunction may influence the efficiency of visuospatial capabilities, while the right-sided hemiparesis, left hemisphere dysfunction can affect motor organization and motor activity. There is a clear link between the right hemisphere, attention deficit disorders and disorders visuospatial skills (Krstic, 2011). For the interpretation of our results certainly have to take into account the important role played by the graphomotor component. Qualitatively speaking surveyed individuals without neurological damage, showed a high degree of cooperation and motivation to work and cooperate with the clear objective aimed at the successful resolution of the task. Surveyed patients after a stroke showed low tolerance to constant error correction, reduced cooperation, quitting of further activities, without a clearly defined purpose. Considering the most comprehensive theory of attention posted by Mesulam, results obtained in this study we could rely on three components of neglect. The first is component of the perceptual neglect and refers to the presence of the interfering informations from the other stimulant field. The other is the motor component, which is an obstacle to direct attention orientation (over attention) towards the neglected side (eyes, limbs of the body). The third is the motivational component, which implies some sort of neglect of the contralateral space, believing that nothing

significant can be expected (Krstic, 2011). Attention deficit disorder is not always the ultimate deficit, he can be contained in a specific visual or verbal modality and accordingly can be damaged when there are lesions of the left or right hemisphere (Lezak, 2004).

In Chanti study that examined the relationship between executive function and walking, and using assessment test employed in our study (TMT A/B), and visual-motor t and time tasks, we get a result that shows significantly more parameters compared to normative values test (Table 2). This explains the difficulties in the simultaneous performance of two or more tasks, the explanation based on the theory on how people handle the received data. The explanation is based on the theory allocation of capacity, considering that the sources of the limited capacity of attention, so performing two tasks that require attention causes poorer efficiency at least in one task. This research is consistent with the results of our research. The results of this research clearly leads us to focus our attention on observation of concept selectivity, limited capacity theory, and "levels" at which its control mechanisms function. Significantly higher values of the parameters in relation to the normative values of the test in patients after stroke and moderately higher values compared to the normative values of the surveyed individuals without neurological damage, with a statistically significant difference in achievement between groups, may indicate a problem in all three or at least one of these control concepts of attention mechanisms (Table 3). In the cognitive functions, perception, memory, and thinking are included. In accordance with the theory of information processing, perception is used for selection, admission, organization and classification of stimuli from the environment, and memory provides encoding and storage tankers of received information. Conceptual reorganization of the acquired information through reviews, provided an adequate response in the form of language, visuospatial behavior or motor activity (Ocić, 1988). Evaluation of cognitive functions comprising level and quality of damage. Cognitive dysfunction can manifest in the form of measurable disturbances in various activities.

In patients with mild stroke can cause subtle language disorders, visuospatial and motor activity. Results of patients after stroke, indicating that two-thirds of patients after stroke had mild cognitive impairment (Table 6), were not unexpected, given that it was hemiparesis and not hemiplegia, which is certainly more difficult clinical condition. This enabled the efficient evaluation of executive functions so that the results obtained which refer to the executive function, are not masked by image of severe cognitive impairment. Within this research, within the group of subjects after stroke, we analyzed the relationship between the level of cognitive impairment and the evaluated parameters walk. The results show that people after stroke with the present cognitive impairment have a lower rate of walking, lower frequency and shorter walking stride length than persons with stroke without cognitive impairment (Table 19). In contrast to the cognitive functions, it is much more difficult to estimate the executive function. Executive functions correspond to the capacity of voluntary activities, or the initiation, planning, self-control and the effective implementation of the various activities (Ocić, 1988). Reduced efficiency of executive functions is expressed through qualitative aspects of behavior. It is believed that the disorders of the executive functions are often present in patients after stroke.

Since the statistical significance we got in assessed relation to cognitive impairment and walk parameters in a group of patients after stroke, depending on the severity of cognitive impairment, the results of assessed relations of executive functions and walk parameters (Table 15), with the same group of respondents allocate executive functions as a separate entity that includes cognitive impairment in itself. The data we obtained using available

literature in a given area for the purpose of comparative analysis, that support the results of our research also suggests that the restrictions may arise from executive dysfunction, requiring new procedural requirements and adjustments in the implementation of medical rehabilitation, in order to increase its efficiency and achieve optimal level of functional independence. Of course, the benefits will be those patients after stroke which no or mild cognitive impairment. Normally, walk is the automatic activity, so long as it performs no attention is paid to the sequence of execution. Give willingly walk, and centers in the CNS responsible for the scheme of walking in humans assume this role.

When damage occurs to these centers, or their routes, these schemes are no longer present, and the individual has to adapt to new circumstances and use schemes available to him. In the case of patients with hemiplegia and hemiparesis, usually it is about pathological or abnormal modified schemes (movement patterns). Kinematic and kinetic aspects of locomotion in these patients vary depending on the severity of the damage, the degree of recovery and the use of various compensatory mechanisms (Pavlovic, 2006). In support of this claim go the results obtained within the framework of a functional assay developments in the field of analysis of spatial and temporal parameters of walking (Table 7). From the results obtained from the evaluation of walk frequency can be seen statistically significant difference in achievement between the surveyed patients after stroke and patients without neurological damage (Table 9). Most respondents had a frequency of walking under the normative values of the percentage representation of 88-92%, within the norms percentages were varied from 8-12%, none of the participants in both groups had a higher frequency of walking from normative values. The reason for slightly lower efficacy in stroke frequency recorded by the patients without neurological damage can be attributed to comorbidity and age. In our most abundant sample examined comorbidity (surveyed in post-stroke patients) was 50% with hypertension, and diabetes mellitus has been represented with 36% (Table 1), the presence of degenerative diseases also not negligible. The results of research that evaluated the frequency of walking in patients with right-sided and left-sided hemiparesis not talk about statistically significant differences achievement as an explanation for the effectiveness of gait parameters can not seek in the lateralization of motor function. However, the results that follow and are related to walking speed (Table 11), and stride length (Table 12), show a statistically significant difference in achievement between the surveyed patients after stroke and patients without neurological damage. Walking speed influences the kinematics, the usual normal walking speed on level ground is 82 m / min. (males have more rapid 86 m / min, women slightly slower 77 m / min). However, the proportion of time spent in different stages of the walk also varies with speed. When a faster walking lunges length increases, so that increasing the speed in proportion leads to an increase in the swing phase, and shortening phase support (Pavlovic, 2006). The results of this study, fully-fitting the above facts that link the spatial and temporal parameters during walking. Surveyed persons after stroke have a lower speed walk from normative values. It is necessary to say that there is no statistically significant difference in gait speed between patients with right-sided and left-sided hemiparesis (Table 11). Following the spatial walk parameter (length lunges), we see that the results obtained in this study indicate a difference in achievement between the surveyed people after stroke and surveyed individuals without neurological damage, as well as the difference between the normative values and the values obtained by measuring the length of stepping in patients after stroke. The person's height, as well as shoes, can affect the size of this parameter, also the width of the surface of the palm and foot progression angle, have an impact on these values.

However, the results obtained in this study does not deny the existence of these impacts, but given the extremely low values of the length of breakthroughs, not all of these factors, or individual, can be qualified as very responsible. Taking into account the results of the evaluated spatial temporal parameters are important for the function of walking, the results of our study are consistent with the facts that rely on the theory of the dynamics of the return of the lower extremities function, through the development phase of recovery ("Movement Therapy in Hemiplegia," Signe Brunnstrom, 1970), (Pavlovic, 2006), which means that the activity as a functional gait in patients after a stroke has to go through all forms of practicing motor control in a standing position on a small area of support, both in the straddle, and the stride (Pavlovic, 2006). The results of this study to assess the trends of the task that is time-limited, show that among the tested individuals after stroke only 20% had normal mobility, 40% had good mobility and 40% have a problem with the movement.

If you look at the percentage representation of movement in patients without neurological impairment in whom 94% had normal mobility, 6% had good mobility, and none of the respondents had problems in the movement, there is an obvious difference of high statistical significance. When surveyed people after stroke, there were respondents who had a risk of falling, as well as those who have requested help or support while moving (Table 14). Bearing in mind the training course in patients after stroke, it must not be overlooked fact preparations for the adoption of this function, directed primarily towards establishing stability in the lower anti-gravity positions, all the way to the stable standing and well-controlled balance and mobility with the time-compliance. Only such an approach is the basis of achieving an efficient, safe, functional and aesthetically satisfying walk (Pavlovic, 2006). One research study that examined the recovery of motor skills of patients after a stroke, came to the conclusion that the improvement in transfer capacity and balance has been seen in exercise tasks with repetition and practicing with a mobile platform, that treatments that allow the recovery of motoric functions are based on high-intensity exercise and repetition exercises of special tasks, focused on mental practice with motor images (Peter et al., 2009). In accordance with the above investigation and observing the results obtained in this study, referring to the possibility of successfully overcoming obstacles with good preservation of equilibrium and balance, surveyed individuals after a stroke had significantly worse results compared to the tested individuals without neurological damage (Table 14) but and in comparison with the normative value. Looking at the results of the surveyed people after stroke and analyzing partial achievements that are an integral part of the overall assessment of the possibilities of overcoming the obstacles, we see that they had a problem with balance, controlling the balance, so that the successful realization (ie. overcoming obstacles) come very rarely. The reason for that may be sought in small number of registered attempts, which is certainly correlated with a small number of successful implementation. When the already mentioned equilibrium and balance, while interpreting the results, it must be taken into account the factor of uncertainty and fear as the following point that makes it difficult to perform a given activity, especially if the patient mobilization hurry or when the motivating factor for the same reduced. Results of a small pilot study that aimed to examine the difference between straight-ahead and curved walking in individuals with the main issues in the movement, supporting the results of our research acquired by assessment of walking and overcoming obstacles in patients after stroke. The test course as the number eight, has been made to present the art of walking in daily life. The results show that the test course as number eight in correlation with walking speed (which is reduced), the length of steps (which are

shorter), with the control of the movement (which is increased), and the fear of falling (that is increased), all the correlations were significant (Hess et al., 2010). In patients after stroke, the following factors should not be overlooked: awareness of their own limitations, willingness to engage in therapy and premorbid personality traits. It can be seen that at an early walk before you gain stability and balance (controlled mobility), regardless of the fact that it can be a huge motivating factor for faster recovery after stroke, there is a great risk for adverse deviation and their compensation and the ultimately may lead to developmental security and lack of functional walking. Based on study of walking recovery in patients after a stroke, it was concluded that the practicing of functional walk should begin the moment the patient is in a standing position and when he receives a satisfactory degree of balance (controlled mobility), regardless of whether it is enough to overcome the swing phase of the affected foot (Pavlovic, 2006). The results of this survey are completely reliant on the above facts from the theoretical framework of the adoption of gait in patients after stroke.

Looking at the results of research speaking of motor skills related to walking function in treated subjects, we have a clear view of the interdependence and coherence of all the analyzed kinematic parameters and kinesthetic walk. This means that in the surveyed people after stroke have a low efficiency in the evaluation of the motor index of the affected lower extremity (Table 13), which affects the efficiency of the functional movement test towards its reduction, further burdening and bringing down the frequency of walking, reducing speed and the shortening stride length. As the final outcome in patients after stroke, we get a reduced efficiency in movement, difficulty in maintaining balance and equilibrium and limited ability of overcoming obstacles (Table 16). Furthermore, they would be faced with all of the above difficulties in situations of reduced efficiency of attention and when the time for completing motor activity is limited. A great part of the research study, talks about the impact and role of cognitive functions on motor recovery and achievement of functional independence, each in their own way and in their own domain. It is believed that executive functions have a stake in motor recovery, but it is not defined if they could be used to predict functional recovery of patients (Nyhus, Barcelo, 2009). Given the above research, we have focused our research on the link between executive function and functional recovery after stroke, showing separately the results from all the evaluated area (executive function, motor function, functional independence) and connecting those results with the intention of discovering and proving existing relationships between them (Table 20). Given that examined patients after stroke with right-sided hemiparesis, showed lower efficiency in planning, organizing and independently perform activities planned, according to the results of our study in which a given functions are positively correlated with functional recovery, the results might be in favor of a slower functional recovery patients with right-hand hemiparesis.

Compatible results with the results obtained in our study of 30 patients with hemiparesis, whose functional recovery was evaluated with Bartel Index - test, which according to the same, patients with left-sided hemiparesis showed significantly better recovery of function self-care, than the patients with right-hand hemiparesis. In addition to association with executive functions, which confirm the results of our research, we must not overlook the fact that the dominant handedness in the general population is 85% of right-handed type, and hence its share in the conquest of functional independence is not negligible. The results of this research provided by the evaluation of the multivariate relationship between two sets of variables, respectively between the indicators of the ability of planned behavior and movement characteristics in patients after stroke (Table 15) show a statistically significant

association. Difficulties that appear in the conceptual organization of the current detection principle in the implementation of activities, time delay or latency between each subsequent response, a number of attempts to start implementation, frequent abandonment of actual activities without a clear insight into the inefficiency, if they focus on the movement can identify the difficulties in initiating motor activity in the presence of a break between each derived motor actions, with lack of anticipation of the next motor action without a clear organization and under the present self-correction. If the basic components of executive function and its properties of the activities ability: the will, planning, premeditated and efficient execution in the presence of cognitive inhibition (Lezak, 2004), the low efficiency of one of the components can affect the ability of efficient movement. The above theoretical fact supports the results of our research that clearly supports the connectivity capabilities of planned behavior and characteristics of the movement. For example, the low awareness of the limitations, as well as the low level of voluntary dynamism can result in a risk of falling. Reduced ability to plan, could result in disorganization of the movement or ineffective action selection, which is characterized by excessive and purposeless effort without successful implementation and without achievement of targeted activities. Ability to plan, means the ability or strategy which will be used to implement intentions and achieve the target with an insight into the real circumstances, with prediction and right choice. It precedes category of formulations intentions, and it is actually the concept of behavior, determined by a purpose. It includes motivation, and insight into their own physical and mental capabilities (Krstic, 2011). Our findings are consistent with the present theoretical review, the examined patients after a stroke had difficulties to perform certain motor activities, we note, as it is important, even in situations where the estimated motor function were not with the major constraints, and therefore requested the necessary outside support, additional stimulation and verbal instructions in order to implement the planned activities. It follows that in our patients after stroke was difficult realization of independent, integrated behavior and performance of target movements. Based on the available literature and previous findings, it is generally accepted that the preservation of executive function, in general, as well as individual aspects, with a special focus on mental flexibility and planned activities may have an effect on motor activity, mobility and integrated behavior (Table 20). Since in the patients after stroke, who have had difficulty with mental flexibility, appeared identical and purposeless movements (Tabela 17), which they did not record as wrong while it was not indicated to them, we can confirm our results with the above statement. It is necessary to clarify that this is not about the appearance of involuntary movements as a result of damage to the pyramid times, but a purposeless movements resulting from difficulties in impulse control and implementation of the planned activities as a result of the low efficiency of executive functions. Reduced effectiveness of all these aspects of executive function, and motor in healthy people may lead to global sluggish, depletion of movement and motor instability (Ocić, 1989). This fact additionally supports the results of this study.

Attention can be seen as a specific example of executive functions. Posner and colleagues (Seligmann et al., 2008), see attention such as an anatomical network whose primary purpose is to influence the work of other brain networks. The same author classifies the attention on *sustained*, which refers to the ability to sustain attention on a task for a certain period of time, *selective*, which allows the reception and elimination of irrelevant information, *divided attention*, which refers to the ability to execute more than one task at the same time; *variable* refers to the rapid change of focus from one task to another. In the study of the same author

(Seligmann et al., 2008), they focused work on divided attention and come to the conclusion that this kind of attention plays an important role in the walk in assigning multiple tasks and changing situations, while the same authors argue that there are clinical implications for the risk of falling. In this study, we evaluated the relationship between attention flexibility and precision of walking in overcoming obstacles in our patients after stroke (Table 17). Flexibility of attention assessed visuo-motor task that required the subjects simultaneously monitor two different conceptual array and switch attention from one to the other, proved to be an effective low. If we could rely on the research of mentioned author, we could define obtained result as weakness in the functioning of the domain variables of attention. It is quite justified in completely healthy people, that when locating a barrier during the walk, they are focusing attention on existing obstacles and overcoming the same, so that at any given moment ignore accuracy in walk (Table 16). The results of our study do not support fully this fact. If we again rely on the above research, the results of this study in patients after stroke, suggesting that they had a good divided attention, but not enough efficient variable attention, and therefore when overcoming obstacles, they had a number of attempts, but many smaller number of successfully implemented, while not neglecting precision during performing activities. If walking is automatic activity and requires no attention, then simultaneously performing additional tasks should not affect the accuracy and speed in the walk, or execution of another task (Hausdorff et al., 2005). Patients walk after stroke is controlled and complex task and has its own characteristics and limitations, so that can not overlook the role of attention during normal walking and walking with obstacles. It is believed that if the source of the limited capacity attention, that is why performing two tasks that require attention cause deterioration, or reduced efficacy in at least one of the tasks. However, when the time between two or more stimuli decreased, the processing time and the implementation of activities will be increased. This theory assumes that it is possible to assign a voluntary capacity to one of the stimuli, or a particular task, even when both tasks disciplined and mostly automatic (Seligmann et al., 2008). This study supports the results of our research, which shows that the evaluated patients after a stroke, will be not focused only on the obstacles, but also the accuracy of the walk, which means that they failed to give priority to one of the activities in terms of increasing the capacity of attention on a chosen activity. Effects of the twin tasks on walking, have been studied in various populations, including healthy young and older adults, as well as patients suffering from neurological diseases. Summing up the results of the given study, it was concluded that the second set task affects walking. Among healthy adults, dual tasks are often caused a decline in the performance of another task. In the case of cognitive and motor task, preference is given to the implementation of cognitive tasks. Within motor task, the problem is most prevalent in walking, a walking speed actually, because it is most vulnerable, at the multiple motor tasks (Table 19). With aging, the presence of such a neurological disease, leads to structural changes in the brain, particularly in the prefrontal area, parts of which are connected with the executive functions and focus systems. Woollacott & Shumway-Cook (Seligmann et al., 2008) based on the results of a number of studies, also concluded that the performance of cognitive tasks during walking, may increase the time of realization of the cognitive task and affect the speed of walking in terms of its reduction, but other changes of a gait pattern were not observed. Another research study, which shows the importance of multiple tasks during walking (Holtzer et al., 2006), is compatible with the aforementioned theory that during walking and performing a cognitive task, there may be changes on the walk, but if you have

multiple tasks from the same or -similar domain, and using the same population of neurons, they will not interfere with one another. Neuroimaging studies (eg, PET, IMRI), showed that during active twin tasks, activity in the prefrontal cortex highlighted, including the inferior frontal gyrus, which is not surprising, as these fields are attributed to executive function. This study confirms the results of this study, because the accuracy and overcoming obstacles during walking, are dual tasks in the same frame, and therefore were not of great mutual influence.

Lundin-Olsson (Hausdorff et al., 2005), in a small study, based on the obtained results, have found that older people who can not at the same time to "walk and talk" fall at the end, while those subjects who can, have far less prone to possible future failures. Springer (Hausdorff et al., 2005), believes that people who fall in idiopathic reasons, increasing variability walk in performing cognitive tasks during walking, while the control group consisted of those who are not prone to fall, did not vary in walking. It is interesting that it is double tasking that connected with the poor showing in tests of attention and executive functions. Faulkner (Seligmann et al., 2008) in a study of 377 elderly subjects, observed that the execution of the double tasks and reaction time associated with the risk of falling. By analyzing the results, they came to the conclusion that minimize the risk of falling in high-risk individuals such as patients after a stroke, can be prevented by reducing the requirements in one of the tasks. Although there are differences in the applied and observed responses in the realization of the double task, the general conclusion is that walking is using attention and effort involved in the double task generally increased as gait becomes less automatic, especially in patients after stroke. Effects of the dual assigning tasks on movements in patients after stroke who have walking impairment due to both (and motor and cognitive deficits) are important, especially when the patient should be involved in the process of medical rehabilitation. The results of this study of patients with post-stroke, suggesting that the movement did not slow down, and that they did not decrease the speed when communicating with the environment (Table 18). Evaluating the relationship between the two sets of variables, and between the indicators of the ability of organizing activity and attention and movement characteristics (Table 17), we found a causal relationship, which means that the low efficiency of the first set of variables, causes low efficiency of the second set of variables. Patients who survive a stroke are experiencing varying degrees of motor and neuropsychological deficiencies.

Although these deficiencies are treated as separate entities in cognitive and medical rehabilitation, there is considerable interaction between them. Certain cognitive functions will not interfere with motor skills, in terms of inhibition or stimulation. The results of this study, supported by an indisputable fact, that in the process of motor recovery, particularly in of mobilization and walking (Table 14), verbal instructions and verbal stimulation are significant, which confirms the fact that communication with the patient is not distracting but stimulating factor. Effects of communication with patients after a stroke, during the walk, are positive and reflected in the achievement of basic safety, resulting in a winning features independent walking and reaching a satisfactory level of operational independence. In addition to the results obtained in our research, came the results of a Dutch research study done on a total of 102 stroke patients in four rehabilitation centers in the Netherlands, and studied the gait, speed walking, general trends in society and came to the conclusion that the walking with company is relevant factor, and of importance for the recovery course of stroke.

Walking in the company had a surprisingly positive effect not only on the speed of walking, but also to maintain balance, endurance during walking, and the use of mobility aids.

Great care was taken in this study is devoted to assess the multivariate relationships between the following variables: indicator of planned behavior ability and the functional independence of patients after a stroke. The results of research show that the tested patients after stroke, exhibit low efficiency in all aspects of planned, purposeful goal-directed behavior (Table 20). Low efficiency dimension additionally joining the low level of attention, especially in the domain of variability, but should not be neglected aspects of sustainability, selectivity and division. Patients after stroke express and motor and cognitive difficulties, which affect the functional training to varying degrees. The results of research studies that examined the interaction between neuropsychological and motor deficiencies (Williams et al., 2008), (Seligmann et al., 2008), suggesting that people after stroke may experience a number of activity limitations. The typical range of these restrictions applies to mobility, communication and self-care. These effects have a negative impact on the movement, but also the social and professional aspects of training. The same research study shows that some patients after stroke typically favor the cognitive function of the motor task and that only certain cognitive functions interfere with motor skills, and the intensity of the motor task will be dosed to achieve the desired motor activity. (Bloem et al., 2008); (Seligmann et al., 2008). In an attempt to detect this relationship, define and implement the process of medical rehabilitation of patients after stroke, we focused on the ability of the activity organization and attention and motor recovery walk, thinking to cognitive and motor impairment can be viewed individually but in the process of rehabilitation treat as a whole. In our group of patients after stroke, low efficiency capabilities of the activity organization exhibited by the difficulty in carrying out the given activity without stimulation and instruction, reduced impulse control, reduced insight into their own mental and motor skills, the concept of behavior is not determined by clear objectives, without being able to predict and finally through the low and independent integrated behavior, maintain and stop the movement, as well as the problem of adaptation. Functional ability is essentially a measure of the achieved level of skills in motor control. Patients after stroke, according to the results of this study did not exceed the limits of their capabilities, among other things because they were unable to assess their ability to limit movement (Table 20). With stimulation, support, verbal encouragement, and instructions of another person, they managed, and the extent of actual motor activity was much higher. The research results also show that this kind of help, in most of the people after a stroke, when they are expected independence, is not reduced. Legitimate need for such help and support was no longer necessary, but the evaluated patients after stroke in our research, managed to free themselves and rely on the approved motor skills. Particularly manifested in those patients after stroke with low effective cognitive abilities. Reduced efficiency capacity planning and organizing activities with all the elements that make up its clinical correlate including low cognitive efficiency are associated with difficulties in communication, socialization and independent functioning (Table 21).

The results of this research show the correlation between the ability of planned behavior and motor skills in patients after stroke, but does not indicate that the effectiveness of the ability of planned behavior can affect the quality of motor recovery in these patients. For the possibility of proving a given impact, it would be necessary to supplement the used battery of tests for the evaluation of this variable and include a larger number of subjects.

Therefore, guided by experienced professionals who have dealt with this problem by monitoring and clinical characteristics of mental competence and motor functioning, with the results of this study, we have opened additional possibilities for a new comprehensive research that would contribute to the understanding of the theoretical aspects of this problem and create the possibility of implementing in therapeutic framework.

CONCLUSION

Based on the results obtained from conducted research, we can conclude that there is a correlation between levels of functioning ability of the activity organization, attention, and motor ability in our patients after stroke. There was also a difference in the level of functioning ability of the activity organization, attention, motor skills with all gait parameters and functional independence among surveyed patients after stroke and surveyed individuals without neurological damage.

It was found that the tested patients after stroke with cognitive impairment, have a lower walking speed, lower frequency and shorter walking stride length than the surveyed patients after stroke without cognitive impairment. It has been found that in patients after stroke in which is recorded the low efficiency in the ability of the planned behavior, ie low efficiency in executive functioning, there is a reduced internal power for movement, and the movement is less manifest. Patients after stroke with low effective attention, slowing movement and losing precision on the gait. It also confirmed the connection between quality of certain aspects of independent behavior, socialization, communication, and locomotion with the quality of executive functions.

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Chapter 65

DIAGNOSIS AND MANAGEMENT OF LANGUAGE IMPAIRMENT IN ACUTE STROKE

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ABSTRACT

Language impairments are frequent in stroke, especially in the hyper acute phase, occurring in 15 to 50% of stroke patients. Recovery from aphasia remains difficult to predict. Language impairment still exists in at least 50% of initially aphasic patients one year after stroke. Post-stroke aphasia is a major source of disability that can lead to impaired communication, reduced social activities, depression and a decreased likelihood of resuming work. The role of the stroke unit in improving morbidity, mortality and recovery has been clearly demonstrated. Nevertheless, the need for intense and sustained acute management of aphasia in specialized stroke units remains controversial. The recent validation of a tool to rapidly screen for aphasia (LAST) after stroke allows for its early detection and management. Early detection of aphasia after stroke may improve outcomes by taking advantage of the synergy between intensive speech therapy and early neural reorganization. Daily re-evaluation will facilitate tailored rehabilitation sessions, multidisciplinary management by the stroke team, and development of educational resources for patients and their caregivers. Standardizing protocols for identifying and managing aphasia requires the co-operation and coordination of the entire stroke team, along with the daily presence of speech therapists. These considerations are crucial for patients in the stroke unit to achieve the full benefit of the management proposed in this chapter, to ultimately promote better long term functional prognosis.

INTRODUCTION

Stroke is a public health issue, representing the third leading cause of death and the first leading cause of disability in adults [1] in the western world. As the population ages, its estimated incidence of 300 to 500/100 000 is expected to increase [2]. Specialized stroke units and/or stroke teams have been created worldwide in order to provide optimized management of stroke in the hyper acute and acute stages. Recent randomized controlled trials (RCT) have proven the efficiency of specialized stroke units in reducing morbidity and mortality, and in facilitating rapid recuperation after stroke [3-6]. Aphasia is a frequent symptom of acute stroke, and a major source of disability. It incurs increased cost of care [7] and has negative economic repercussions, such as decreased return to work [8,9]. However, early intense and sustained management of aphasia in stroke units remains controversial.

We share herein our experience of the early identification and treatment of aphasia after stroke, based primarily on a neurolinguistic framework.

We further describe existing protocols and discuss the expected benefits of early management. Optimal standardization of aphasia management can be achieved in stroke units with collaboration among physicians, nurses and speech therapists, resulting in improved outcomes after stroke. Moreover, clinically-driven research in aphasia in the acute setting is feasible and essential to improving recovery and rehabilitation outcomes.

1. IMPAIRMENTS IN THE ACUTE PHASE OF STROKE: GENERALITIES

Survivors of stroke often experience multiple co-occurring impairments, such as hemiplegia [10,11] dysphagia [12, 13], incontinence [14,15] depression [16], cognitive changes [17,18], dysarthria [19,20] and/or language impairment [21,22]. Second to hemiparesis, dysphagia, dysarthria, and language impairment are the most frequent [20]. The incidence of dysphagia approximates 55% in the acute stage [12,13].

Dysarthria and language impairment are almost as frequent in the acute phase of stroke, occurring in approximatively one-third of all stroke patients. Nonetheless, these impairments are probably underestimated, particularly in the hyper acute stage, given the lack of standardized assessments and the potential for rapid recovery [21, 23-25]. Dysarthria may occur as isolated speech impairment or with concomitant aphasia. Studies have reported dysarthria to range from 8 [26] to 42% [19] and aphasia to range from 15 [24] to 41% [12] following stroke.

Inclusion of different stroke etiologies, first versus recurrent stroke patients and different methodologies likely contributes to variations in reported frequencies of language impairment.

1.1. Definitions

Stroke is usually defined as a sudden onset of signs of focal disturbance of cerebral function lasting over 24 hours [27]. Language impairment literature can relate to ischemic, intracerebral hemorrhage (ICH) or subarachnoid hemorrhage (SAH) stroke etiologies.

Among stroke subtypes, ischemic etiology is the most frequent cause of stroke, followed by ICH and then SAH [28-30]. While there is typically an abrupt onset of neurological deficit after ischemic stroke, a progressive evolution, often with considerable tissue displacement [31], can also exist after ICH [26]. In addition, there is as much as a 10 fold higher frequency of decreased level of consciousness after ICH compared to ischemic stroke [26]. Consequently, aphasia can be identified very early after ischemic stroke, while patients with ICH may require ongoing monitoring for adequate identification of its presence. After ischemic stroke, some patients may fully recover from aphasia within days of stroke onset [24], thereby warranting careful monitoring for early recovery. Aphasia and other focal neurological deficits are not generally expected to occur after SAH, except occasionally as transient signs of vasospasm [30], or after aneurysm clipping [32].

Aphasia or language impairment is defined as an acquired impairment, characterized by anomia [33] and/or other deficits in expressive or receptive language, evidenced in any modality: speaking, listening, reading, or writing [34]. We use a neurolinguistic framework to describe brain behavior relationships in the identification and management of language impairment after stroke. (Figure 1)

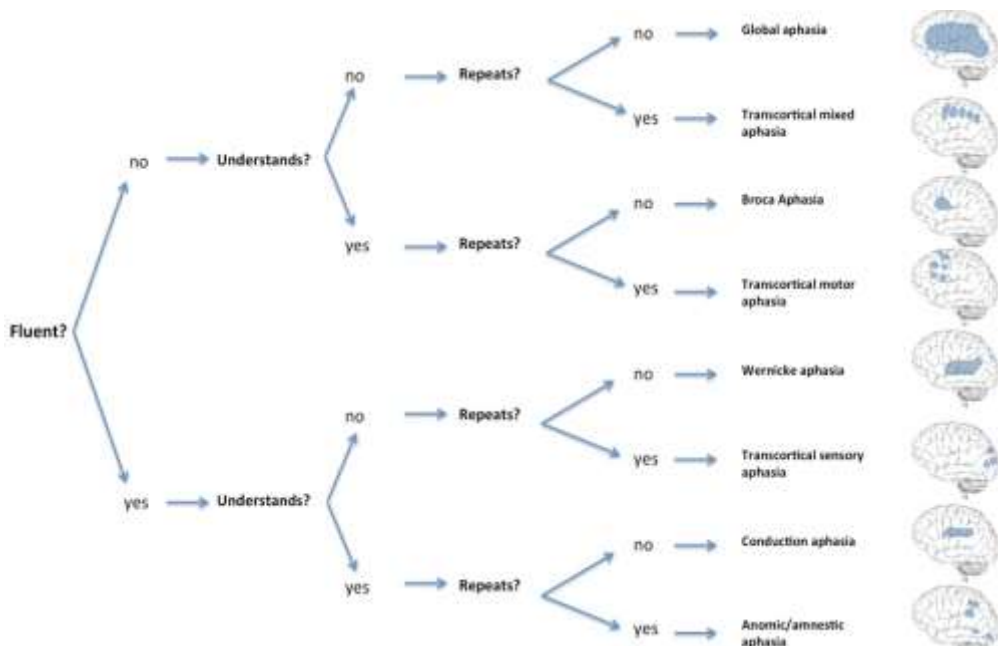


Figure 1. Decision-making tree in aphasic patients.

1.2. Incidence, Predictors and Phenotypes of Language Impairment

Aphasia results from injury to the dominant hemisphere, which involves the left hemisphere in right-handed patients and in 70% of left-handed patients [35,36].

Table 1. Frequency of Acute Aphasia according Stroke Etiology, by increasing frequency

Study	Frequency %	Sample n	Assessing Health Professional
MIXED ETIOLOGIES			
All Etiologies			
Hilari* (2011)	17	12	unclear
Hier et al ^a (1994)	21	1805	neurologist
Inzitari et al (1998)	22	339	physician
Kumral et al ^{a,b} (1988)	23	2000	neurologist
Kyrozos et al* (2009)	23	555	physician
Lawrence et al* (2001)	23	1259	physician
Bersano et al (2009)	28	8848	neurologist
Stegmayr et al* (1994)	30	3542	medical staff
Sturmer et al ^b (2002)	31	270	physician
Dickey et al ^c (2010)	35	3207	stroke care team or CNS
ISCH and ICH			
Hier et al (1994)	23	1510	neurologist
Croqueolois & Bogousslavsky* (2011)	26	5880	physician
Hilari* (2011)	33	96	unclear
Laska et al (2001)	33	106	SLT
Bogousslavsky et al* (1998)	34	1000	unclear
Guyonard et al ^d (2009)	41	2983	physician or nurse
ICH and SAH			
Hier et al ^e (1994)	9	490	neurologist
Sturmer et al (2002)	20	50	physician
ISOLATED ETIOLOGIES			
ICH			
Babou ^f (2009)	15	100	physician
Hier et al (1994)	16	237	neurologist
Croqueolois & Bogousslavsky* (2011)	26	647	physician
Bersano et al (2009)	32	1009	neurologist
Guyonard et al (2009)	36	314	physician or nurse
SAH			
Hier et al ^g (1994)	3	243	neurologist
Anderson et al ^h (2006)	12	868	neuropsychologist
ISCH			
Inatomi et al ⁱ (2008)	15	835	neurologist or SLT
Lubart et al ^{a,b} (2005)	23	140	neurologist
Hier et al (1994)	24	1273	neurologist
Croqueolois & Bogousslavsky* (2011)	26	5233	physician
Engelter et al* (2006)	30	269	neurologist
Sturmer et al ^b (2002)	34	220	physician
Hilari* (2011)	39	76	unclear
Guyonard et al (2009)	40	2318	physician or nurse

CNS= Canadian Neurological Scale.; ICH=Intracerebral Haemorrhage; ISCH=Ischemic Etiology; SAH=Subarachnoid Haemorrhage

SLT=Speech Language Therapist; * first ever stroke.; (a)proportion of SAH higher than that of ICH.; (b)TIA included in ischemic category.(c)35% incidence documented at discharge, compared to 30% very early after stroke;(d)included ISCH, ICH, and undetermined etiology;(e)Excluded cerebral trauma as cause of ICH;(f)Used a cued word finding task; combined results for those with hypothermia and those with normothermia; (g)Only included patients with good premorbid activities of daily living; (h)Geriatric ward sample of older patients.

The neural network subserving language function is increasingly recognized to be a large and complex system involving regions throughout the cerebral hemisphere. The language network comprises areas of the perisylvian cortex, including the classical language areas of Broca and Wernicke: Broca's area or Brodmann's area 44 in the posterior inferior frontal gyrus innervates adjacent motor neurons subserving the mouth and larynx, and controls the output of spoken language; Wernicke's area or Brodmann's area 22, comprising the posterior two-thirds of the superior temporal gyrus, receives information from the auditory cortex and accesses a network of cortical associations to assign word meanings; the angular gyrus in the inferior parietal lobule is adjacent to visual receptive areas and subserves the perception of written language, as well as other language processing functions. Additional regions have a significant contribution to language function: the insula, several frontal and temporal lobe regions and vast regions of the temporal, occipital, and parietal cortex, as well as subcortical nuclei. [37-42]

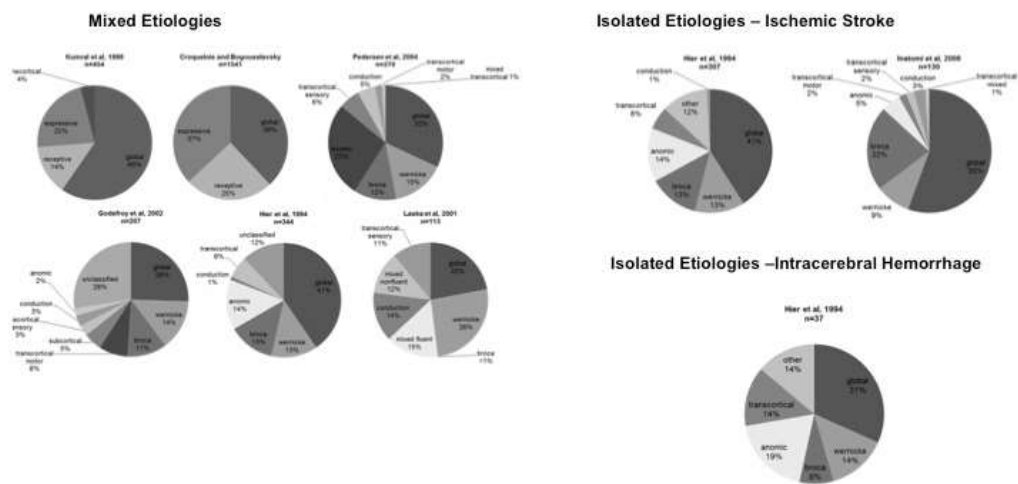


Figure 2. Classification of aphasia subtypes according to stroke etiologies by study. Total percentages may not equal one hundred, due to rounding to the nearest percent.

The incidence of aphasia may differ according to stroke etiology (see table 1). Some studies have documented aphasia in unselected stroke samples, involving all three etiologies: ischemic, ICH and SAH, with frequencies ranging between 21% [43] and 35% [44]. Samples that considered only first-ever stroke for all three etiologies have reported frequencies ranging from 17 [45] to 30% [46]. When SAH was excluded, studies have reported frequencies ranging from 23 [43] to 41% [12]. To our knowledge, only two studies have included both types of hemorrhagic stroke, reporting a combined frequency of 9% [43] and 20% [47]. After isolated ICH, the reported frequency of aphasia ranges from 15 [48] to 36% [12]. Two studies have reported aphasia frequencies of 3 [12] and 12% [31] after SAH. Documents of the frequency of aphasia after ischemic stroke range from 15 [24] to 40% [12], with frequencies ranging from 23 [20] to 39% [37] after first ischemic stroke. The presence of language impairment is preferentially associated with previous stroke [12], increasing age [12,21,22,41,42] female gender [42], stroke severity [24], atrial fibrillation [21,22,49,50], cardio-embolic etiology [51]. Published reports differ concerning the frequency of

phenotypes of aphasia after stroke and there is a paucity of literature describing the clinico-radiological correlation. Croquelois and Bogousslavsky recently studied 1541 patients with aphasia in the early stages of stroke (ie within the 24 hours following onset), classifying them according to aphasic patterns, where 38% presented with expressive-receptive aphasia, 37% with expressive aphasia and 25% with receptive aphasia [52] (see figure 2). In same setting, Kumral and his colleagues described a series of 454 patients with acute aphasia in the first four weeks after stroke, identifying receptive-expressive aphasia in 60%, expressive aphasia in 22%, and receptive aphasia in 14% [30]. Two studies have described more specific phenotypes in series of consecutively admitted patients with aphasia [23, 25] (see figure 2), where one demonstrated the highest frequency for expressive-receptive aphasia [23] and the other for expressive aphasia [25]. In both studies, receptive aphasia types were least frequent [23,25]. Two additional studies have reported subtypes of aphasia in acute stroke patients following ischemic stroke and ICH, excluding SAH [43, 53]. In one of these two studies, expressive-receptive aphasia predominated [43] at 40%, while receptive aphasia was most frequent in the other [53] at 65%.

The small sample size in the latter study may account for this variation [53], when compared to the larger studies. Three studies have considered aphasia classifications after isolated etiologies. Two documented aphasia after ischemic etiology [24,43], demonstrating that expressive-receptive aphasia predominated, with frequencies of 41% [43] and 56% [24], followed by expressive aphasia with frequencies of 27% [43] and 29% [24]. Receptive aphasia was least frequent at 14% in both studies.

Hier and his colleagues also documented aphasia subtypes after intracerebral hemorrhage, demonstrating the highest frequency for receptive-expressive aphasia at 31%, followed by expressive aphasia at 27% and then receptive aphasia at 14% [43].

Discrepancies in these results are probably due to differences between the sensitivity and specificity of tools versus bedside clinical evaluation and to the timing of evaluation, as aphasia can improve rapidly shortly after the onset of stroke. To illustrate, a study involving 130 acute ischemic stroke patients with aphasia demonstrated improvement of aphasia in 46% and resolution in 21% within the first 10 days following stroke onset [24]. In addition to reporting aphasia based on subtypes in these studies, some authors noted numerous unclassifiable aphasias (25%) [23, 43] or subcortical aphasias [23]. These unclassifiable aphasias may be comprised of patients with co-occurring subtypes or they may result from the method of determining subtypes, where certain patients do not fit the study's definition of each subtype. Also, diaschisis and "penumbra phenomenon", resulting in hypoperfusion to anatomical regions associated with aphasia, are possible explanations for rapid improvement, subcortical aphasia [23] or for evolution from nonfluent to fluent aphasia [25].

1.3. Prognosis

In spite of the potential for rapid improvement, language impairment usually still affects 50% of initially aphasic patients 18 months after stroke [25,53], thereby contributing to altered quality of life beyond the aphasia itself [45]. Primary consequences may include symptoms of depression, difficulty to participating in rehabilitation, difficulty understanding therapists', social draw-back and decreased frequency of professional activities [54-56].

It is difficult to predict the outcome of aphasia, even if aphasia after hemorrhagic stroke has a better prognosis than after cerebral infarctions [57]. To the present day, only initial stroke severity (according to NIHSS) and the initial severity of aphasia have been shown to predict importance of aphasia recovery after acute ischemic stroke [24,25]. In a recent study, Maas conclude that advanced age, the presence of hypodense regions, a history of previous stroke, and sedentary activity level significantly predicted a diminished likelihood of language improvement at discharge. At 6 months of follow-up, only the prestroke mRS significantly impacted likelihood of improvement [58].

2. TOOLS FOR LANGUAGE ASSESSMENT

2.1. Available Tools

Early detection of aphasia after stroke may improve rehabilitation by taking advantage of the synergy between intensive speech therapy and early neural reorganization [59-61]. Tools capable of detecting aphasia and evaluating its severity during the acute phase of stroke might help to improve early rehabilitation and to predict outcome [62]. Standard aphasia rating scales such as the Western Aphasia Battery, the Boston Diagnostic Aphasia Evaluation (BDAE), and the Boston Naming Test are not appropriate for use during the acute phase of stroke [61,63-65]. In particular, these gold standard test batteries take too long to complete and must be administered by speech and language therapists [63-65]. Global stroke rating scales such as the National Institutes of Health Stroke Scale and the Scandinavian Stroke Scale include language items and have been developed for use in acute settings [66-71], but they do not reliably detect aphasia [62]. Several attempts have been made to develop and validate brief aphasia screening scales suitable for patients with acute stroke [72-79], but all have inherent structural limitations, including [61]: (1) inclusion of written language subtests, the results of which are influenced by hemiplegia and illiteracy [59,73-76,79]; (2) use of complex visual material inappropriate for stroke patients with neuro-visual deficits [73,74], (3) inclusion of subtests that are markedly influenced by attention/executive dysfunction [73,74]; (4) excessively lengthy administration [76]; (5) difficulties with administration or scoring [59,72,76,79]; and (6) IQ dependency [75]. Some of these scales also have poor sensitivity for the detection of aphasia and provide little information regarding measures of validity and reliability [59,76].

2.2. A Novel Tool: LAST, the Language Screening Test

Early and precise diagnosis of aphasia is necessary and justified for informed decision making, such as the use of thrombolysis and the implementation of early and individually-tailored rehabilitation. Evaluation may help to localize lesion site, prior to magnetic resonance imaging [80]. A quantitative tool is needed to document the risk or presence of aphasia and to quantify repeat evaluations. Such a tool would also permit description of aphasic patterns according to lesion localization, such as transcortical aphasia following acute border-zone infarcts [81,82]. A recent study investigated the correlation between language tasks and brain

lesions, concluding that word repetition, sentence repetition and naming best-predicted lesions in the left hemisphere lesion [83]. Counting and comprehension were also impaired in patients with brain injuries [83].

We recently developed and validated a brief language screening scale in French, the Language Screening Test (LAST) [84], to screen for risk of aphasia and to facilitate timely assessment of patients after acute stroke. We validated and documented reliability for two versions of the tool in an unselected sample of chronic and acute stroke patients, excluding SAH. The scale consists of 5 subtests: picture naming (items selected based on lexical familiarity and image evoking value), repetition (of a word and a sentence), automatic sequence (counting from 1 to 10), picture recognition (including semantic, visual and phonemic foils) and auditory commands (including 1 simple and 2 complex instructions). These subtests were chosen for their high correlation with left hemisphere lesions. Two parallel and equivalent versions of LAST (a and b) were developed to avoid a re-test effect. Each item is scored as 1 if correct and 0 if incorrect or inappropriate; The total scores range from 0 to 15, with a cut-off at 14 for the diagnosis of aphasia, as compared to the BDAE. The test is administered on a letter-sized sheet of paper (one side for the expressive index and one side for the receptive index). Administration requires fewer than three minutes. The simplicity of LAST makes it suitable for bedside administration by any health professional. The LAST is useful as a rapid and reliable screen for aphasia, with proven specificity. Given the existence of two parallel versions, a second quantification of aphasia is possible during a follow-up period.

3. RECOVERY

Speech and language therapy is assumed to be beneficial; A recent Cochrane review (2010) reported some indications of the effectiveness of SLT for people with aphasia following stroke [85]. Moreover, the authors documented results favouring intensive SLT over conventional SLT. However, there was insufficient evidence in this review to establish the effectiveness of one SLT approach over another [85].

Neural processes underlying recovery remain unknown [86]. Different factors may play a role, such as age, stroke size and location, premorbid level of education, and environmental support [63]. It has been documented that hemorrhagic strokes evolve better than ischemic strokes [57]. However, the only predictive factor of speech and language recovery following cerebral infarctions is the initial severity of stroke [24,25]. One study postulated that, as with motor recovery, improvement from aphasia is directly correlated with the initial deficiency, with an estimated recovery of 70% at 90 days post stroke onset [87]. Aphasia usually results from lesions affecting the left hemisphere in right-handed patients as well as in the majority of left-handed patients [35,36]. Cerebral plasticity and changes in neural organization interfere with the restoration of language function [88,89]. This is due to post-stroke compensation in the undamaged language areas, perilesional tissue and homologue right language areas [90]. In the setting of a very focal lesion, perilesional areas participate in recovery at the acute phase and during rehabilitation. Two different mechanisms are supposed: transient hypoperfusion of these territories (“penumbra”) and early neuronal reorganization with up regulation of these areas [91]. In larger lesions, language recovery

could implicate the right hemisphere, as demonstrated in functional imaging and neurophysiologic studies [92-94]. Homologous right-sided regions that could be implicated in the process of language recovery are the superior temporal lobe (auditory control), the inferior frontal gyrus, pre-motor areas (planning of motor actions and nonlinguistic perception of language) and the motor cortex (execution of verbal motor actions) [86,95,96]. A recent study reports the extraordinary case of a man who benefited from a pre and post stroke functional MRI (fMRI): the patient presented with a Broca's aphasia after a large ischemic infarction within the territory of the left middle cerebral artery, with almost complete recovery [96]. The fMRI shows a perilesional reorganization but no significant recruitment of the right hemisphere one year after stroke [96]. A second functional MRI study reported the dynamics of language reorganization in 14 stroke patients describing activation of perilesional areas initially (as explored at day 2) [90]. Subsequent areas of reorganization included the right homologue of Broca's area in the sub acute period (day 12 after stroke) and later the reactivation of the left language areas in the chronic phase (explored one year after onset of symptoms) [90]. These findings should be taken in account when planning rehabilitation.

4. REHABILITATION

Speech and language therapy (SLT) aims at improving communication using verbal or nonverbal treatment modalities. Recent guidelines recommend speech therapy for patients with aphasia following stroke in spite of contradictory results from a few previous studies [85,97,98]. This heterogeneity could result from unsuitable tools, large heterogeneous cohorts, initiation of intensive therapy at differing time periods following stroke [99] or different rehabilitation techniques [98,100].

Functional imaging and behavioral studies have shown that SLT can modulate neural plasticity [101]. Because inappropriate rehabilitation may interfere with reorganization of language, it should be implemented according to the aphasia classification. Therefore, semantic phonological, and/or morphosyntactic therapies should be proposed according to the initial language impairment [102, 103]. In our center, different methods of SLT are performed according to each patient's needs and type of aphasia. We suggest that therapy should be implemented on a daily basis as early after stroke onset as possible. In particular, the specific contributions of the right hemisphere, such as prosodic and rhythmic properties of language, should be targeted in therapy to favor activation of contralateral language areas [104]. Early SLT in nonverbal patients or patients with severely reduced verbal output is important in order to avoid stereotypies. In accordance with one hypothesis on the dynamics of language recovery, and to capitalize on the potential role of the right hemisphere, we practice early SLT using melodic intonation therapy (MIT) [92]. We have proposed an auditory stimulation program, using a daily music listening program in our unit (one hour of music with French lyrics for our francophone population), derived from recently reported benefits [104]. Patients are encouraged to listen to music with lyrics and to actively sing along. A recent study with clinician blinding has sought to determine the immediate effects of introducing MIT early after stroke onset in patients with Broca's aphasia [105]. The treatment group showed a significant improvement compared to the control group, supporting the benefit of using this therapy method after acute stroke [105].

Although some have argued that early speech therapy is not feasible or necessary, we contend that it is indeed feasible and paramount to optimizing recovery. Second to promoting language recovery, initiation of therapy may reassure patients and help educate caregivers. As part of the therapeutic initiative, caregivers are provided advice for supportive communication [106] and to aid in eliciting compensatory strategies from the patient. Early therapy also facilitates better communication in patient care regarding medical explanations, instructions, and interactions with other therapists and the medical team.

Contribution from the entire medical and paramedical team is essential in the treatment of aphasia. In our stroke unit, therapeutic information is discussed with nursing staff, because their frequent patient interactions are crucial for progression toward rehabilitation goals. Their numerous daily interventions with patients may play a role in increasing the intensity and functionality of speech and language objectives. Providing adequate information about aphasia to front-line health professionals and families is essential to avoid ambiguous interactions. This will promote clear explanations conducive to the reception capabilities of patients. Similar educational information and explanations are necessary for both families and caregivers to avoid misconceptions. For example, some relatives may think that a patient has incurred hearing loss, amnesia, cognitive decline or dementia, psychiatric disorders or that the patient lacks physical sensation or emotion. Unfortunately, aphasia is not well understood, and educating family members and caregivers is essential to facilitate faster and more efficient recovery on the part of patients. Families and caregivers need to be well informed about the diagnosis and potential prognosis of the aphasia. As with front-line health care workers, other health professionals, such as physiotherapists or occupational therapists are inherently implicated in language rehabilitation in the acute phase. Their actions should be verbalized in an accessible formulation, with clear articulation, and with sufficient confirmation that the patient understands the instructions. In patients who are nonverbal or who have extremely limited verbal output, compensatory strategies such as the use of pictures, gestural communication, and letter require explanation to families and caregivers to prevent communication breakdown. Associated impairments, such as anosognosia after posteriorly localized aphasia and depression after anteriorly localized aphasia require additional considerations and caregiver education from the stroke team.

5. PERSPECTIVES

Effectiveness of SLT has been shown in different studies, and tools are now available to assess aphasia in acute stroke. Randomized controlled trials are still needed to indicate the best approach to delivering speech and language therapy. This will inform indications for the optimal approach, frequency, and duration of allocation and format of SLT for specific patient groups. Fortunately, recent randomized controlled studies have been conducted in the acute stage with a particular consideration of methods of speech therapy [103,107]. Controversy from potentially contradictory results arose from methodological differences such as group size, randomization methods, and type, timing (early vs. delayed) and frequency of therapy (intensive vs. conventional) [85, 98,108]. Intensive SLT, of any type, has been shown to be beneficial according to some authors [103]. Others consider that

“intensive” and “early” SLT should be undertaken to promote early neuronal re-organization [59-61,103].

Considering different therapy types by SLTs, a recent RCT investigated the effect of “Language enrichment therapy” [107], starting at day 2 post stroke and continuing to day 21. The control group received no SLT for at least the first 21 days post stroke. At day 21, 58% of the treatment group achieved a higher score on the aphasia test battery, while 35% achieved higher scores in the control group. However, the treatment group did not maintain gains in verbal communication six months following stroke. A more recent pilot RCT evaluated daily SLT according to patient classifications of aphasia, involving linguistically driven therapeutic tasks [103]. They compared patient tailored therapy with usual care and found that patients in the treatment group had significantly higher scores on the aphasia quotient and on a functional communication measure than the usual care group. Additional RCTs are still needed to better determine the optimal type, timing and intensity of speech language therapy in the setting of acute stroke.

The validation and availability of the LAST and the recent demonstration of the feasibility of conducting RCTs in the very early stages of stroke provide the impetus for designing future randomized studies that take type, timing and intensity of SLT into account after acute stroke. In addition, use of a validated language scale, such as the LAST, in randomized trials or consecutive cohorts, could further promote research correlating acute clinical signs of aphasia with MRI, by which the efficacy of therapy for aphasia subtypes could be documented.

CONCLUSION

The early detection of aphasia after stroke is essential for reliable diagnosis in order to optimize rehabilitation. Available recent tools now allow for rapid and precise assessment of aphasia. Standard care protocols and teamwork promote best practice in the use of these tools, rendering them more efficient and effective. The implementation of such protocols involves the cooperation and coordination of both medical and paramedical personnel and the daily presence of speech therapists in stroke units. In particular, we recommend this daily presence for patients with aphasia, so that they may derive maximum benefit and the advantages of units that provide this type of standard care. Consequently, we expect that the long-term functional prognosis of these patients with aphasia will improve. Moreover, future randomized trials are essential to develop specific interventions for and to optimize SLT support.

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Chapter 66

SOCIO-ECONOMIC BURDEN OF MYOCARDIAL INFARCTION AND STROKE ATTRIBUTED TO ROAD TRAFFIC NOISE IN EUROPE

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ABSTRACT

This study conducted in 2016 aimed to update and extend the existing burden of disease calculations of the WHO for road traffic noise-attributed myocardial infarction and stroke. All analyses were limited to 24 European countries and the year 2012. The burden of road traffic noise-attributed myocardial infarction and stroke was expressed in disability-adjusted life-years (DALYs) and their corresponding monetary value. Noise exposure data on agglomerations with more than 100 000 inhabitants were elicited from the Noise Observation and Information Service for Europe. We used an updated linear risk of myocardial infarction based on eight estimates from cohort studies and newly generated linear and categorical risks of stroke. Morbidity/mortality data for acute myocardial infarction and cerebrovascular disease were extracted for 474 million Europeans. Results showed 84 772 DALYs and € 2.25 billion lost to road traffic noise-attributed myocardial infarction. The burden of stroke varied from 20 608 DALYs and € 550 million (linear trend) to 202 223 and € 5.36 billion (non-linear trend). This study reported preliminary evidence regarding the DALYs due to road traffic noise-attributed myocardial infarction and road traffic noise-attributed stroke in Europe, which were associated with considerable socio-economic burden.

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1. INTRODUCTION

Cardiovascular disease is a leading morbidity and mortality cause worldwide, culpable for about 30% of all deaths, and its burden is projected to increase globally until 2020 [1]. These fatal cases are mostly due to myocardial infarction and stroke, which were fourth and fifth causes for global years of life lost in 1990, and now hold first and third places, respectively [2]. Whereas mortality rates are gradually decreasing, the number of hospitalizations due to cardiovascular disease is increasing [3]. There is also gross variation in burden of cardiovascular disease across European countries [3]. However, classical risk factors cannot fully explain the variance in cardiovascular disease risk [4-6].

Road traffic noise is now widely accepted as a cardiovascular disease risk factor. Acting as an environmental stressor, it has adverse impact on catecholamine and cortisol levels, vascular hypertonicity, endothelial dysfunction, sleep quality and metabolic homeostasis, which may ultimately lead to hypertension, myocardial infarction and stroke [7]. Noise is certainly not as prominent cardiovascular disease risk factor as some lifestyle and behavioral influences, but since individual-level interventions are clearly not sufficient to reduce the global burden of cardiovascular disease, mitigating noise exposure within cities may provide substantive benefit on ecological scale, as it will affect majority of the population.

In 2011, the WHO estimated an annual loss of 60 768 disability-adjusted life-years (DALYs) from road traffic noise-attributed ischemic heart disease in Western Europe, or approximately 1.8% of the total burden of ischemic heart disease in high-income European states in the year 2004 [8]. Such quantitative measures of burden of disease are anchor elements for environmental health policy and decision making, and for adequate resource allocation and evaluation of interventions to reduce road traffic noise exposure. These calculations, however, involved some unavoidable assumptions and uncertainties, which can now be corrected. Therefore, the present study aimed to update and extend WHO's burden of disease calculations for road traffic noise-attributed myocardial infarction and to generate estimates of the burden of road traffic noise-attributed stroke.

2. METHODS

2.1. Burden of Disease Estimation

The burden of road traffic noise-attributed myocardial infarction and stroke was expressed in DALYs, which represent "years of life lost to premature death and years lived with a disability of specified severity and duration" [9]. Our analyses draw on the accepted WHO methodology previously applied to road traffic noise and myocardial infarction [8, 10]. Since our study used official online resources and ecological data, it did not undergo ethics review by an Institutional Review Board.

The first step was to calculate population-attributable fraction (PAF) for each disease: $PAF = \{\Sigma(P_i * RR_i) - 1\} / \Sigma(P_i * RR_i)$, (Equation 1), where “ P_i ” is the proportion of the population in noise exposure category “ I ”; “ RR_i ” is the relative risk of the disease at exposure category “ I ” compared to reference level; where “ ΣP_i ” should be equal to 1.

PAF was then multiplied by the number of non-fatal cases during the year 2012, in order to determine the number of non-fatal cases attributed to road traffic noise. Next, we estimated the number of years lived with disability (YLD) due to these cases: $YLD = I * DW * D$, (Equation 2), where “ I ” is the number of non-fatal cases attributed to road traffic noise; “ DW ” is the disability weight for the respective diagnosis; and “ D ” is the duration of disability in years.

The number of years of life lost (YLL) due to the fatal cases was computed by multiplying the number of deaths by the standard life expectancy at the age at which death occurred. This was done separately for men and women, after which the total number of YLL for each gender was multiplied by PAF to get YLL attributable to road traffic noise. Finally, the number of DALYs was estimated by adding together YLL and YLD.

We extended the WHO’s methodology by assigning monetary value to these DALYs, in order to provide more straightforward and intuitive interpretation of the burden of disease. This was done by multiplying the DALYs by the gross domestic product (GDP) per capita in EU-28 for the year 2012 [11]. All data were stored into spreadsheets and analyzed with Microsoft Excel v. 2010.

2.2. Noise Exposure

Information regarding the percentage of residents of major agglomerations (> 100 000) in Europe exposed to different levels of road traffic noise in 2012 was extracted from strategic noise maps available through the most thorough source so far – the Noise Observation and Information Service for Europe (NOISE) [12]. Since the Noise Observation and Information Service for Europe does not report the number of people exposed to < 55 dB, it was estimated by subtracting the number of people in the reported categories from the total population covered in the noise maps. The noise indicator we used was the day-evening-night noise level (L_{den}). Noise maps have been prepared according to the European Noise Directive [13]. Some of EU-28 member states (e.g., Greece, Hungary, Slovakia, Slovenia, and Latvia) did not provide relevant data and were excluded from the analyses.

2.3. Risk Estimates

We assigned relative risk estimates to the midpoint of each 5-dB road traffic noise category. The risk estimates used in the WHO’s calculations [8] were generated by a polynomial function based on analytic studies (cohort and case-control) [14]. In our analyses, we wanted to provide more robust estimates; therefore, we relied only on estimates from cohort studies, which was not feasible at the time of Babisch’s first meta-analysis. Since then, new high quality evidence was published. We obtained a robust linear risk of myocardial infarction (RR per 10 dB = 1.065, 95% CI: 1.007 – 1.127), using the seven cohort risk estimates extracted by Babisch in his updated 2014 meta-analysis [15], along with the risk

estimate from a cohort study just-published at the beginning of 2016 [16]. (See Table 1) They were pooled together under the inverse variance heterogeneity estimator, a meta-analytical model which is superior to the random effects estimator used by Babisch [14]. This novel estimator favors studies with lowest probability of random error, it has a more conservative 95% CI, retains a correct coverage probability and exhibits a lesser true variance regardless of heterogeneity [17]. Vienneau et al. found in their extensive meta-analysis that the exposure-response relationship between traffic noise and myocardial infarction did not deviate significantly from linearity [18], therefore, we treated the risk of myocardial infarction as linear.

With respect to stroke, few studies have been published overall, let alone cohort studies. All studies were synthesized by Dzhambov and Dimitrova [19], who pooled six estimates (three cohort, two cross-sectional, and one ecological) yielding RR per 10 dB = 1.010 (95% CI: 0.962 – 1.061). Of note, the authors found evidence against the linearity of this exposure-response relationship and reported a polynomial approximation ($R^2 = 0.95$) of the non-linear risk, based on one ecological and three cohort estimates: $RR = 0.00066295186571 \cdot (L_{den})^2 - 0.06779154098343 \cdot (L_{den}) + 2.73847143171073$ (Equation 3). The linear and the non-linear risks of stroke were both used as alternatives in our burden of disease analysis.

Table 1. Individual cohort study risk estimates and pooled linear risk for myocardial infarction per 10 dB increase of road traffic day-evening-night noise level

Study	RR	95% CI		Weight (%)
Caerphilly-CO*	0.969	0.727	1.290	1.93
Speedwell-CO*	0.875	0.683	1.121	2.58
Netherlands-CO*	1.009	0.941	1.082	32.48
Vancouver-CO-Male*	1.150	1.050	1.260	19.05
Vancouver-CO-Female*	1.110	0.990	1.230	13.44
Copenhagen plus-CO-Male*	1.140	1.030	1.260	15.59
Copenhagen plus-CO-Female*	1.060	0.910	1.230	6.97
Bodin et al. (2016)	0.990	0.860	1.140	7.97
Pooled RR per 10 dB	1.065	1.007	1.127	100
Heterogeneity statistics:				
I ²	37.525	0.000	72.406	
Cochran's Q	11.205			
Chi squares p-value	0.130			

Note. The meta-analysis is conducted under the inverse variance heterogeneity model. *These studies are labelled according to Babisch [16], and the corresponding citations are Caerphilly-CO and Speedwell-CO: Babisch W, Ising H, Gallacher JEJ, Sweetnam PM, Elwood PC. Traffic noise and cardiovascular risk: The Caerphilly and Speedwell studies, third phase-10 years follow-up. Arch Environ Health 1999; 54:210-16.; Netherlands-CO: Beelen R, Hoek G, Houthuijs D, van den Brandt PA, Goldbohm A, Fischer P, Schouten LJ, Armstrong B, Brunekreef B. The joint association of air pollution and noise from road traffic with cardiovascular mortality in a cohort study. Occup Environ Med 2009; 66:243-50.; Vancouver-CO: Gan WQ, Davies HW, Koehoorn M, Brauer M. Association of long-term exposure to community noise and traffic-related air pollution with coronary heart disease mortality. Am J Epidemiol 2012; 175:898-906.; Copenhagen plus-CO: Sørensen M, Andersen ZJ, Nørsgård RB, Jensen SS, Lillønd K, Beelen R, Schmidt EB, Tjønnelund A, Overvad K, Raaschou-Nielsen O. Road traffic noise and incident myocardial infarction: A prospective cohort study. PLoS ONE 2012; 7:1-7.

2.4. Morbidity, Mortality and Life Expectancy

Incident myocardial infarction and stroke cases (morbidity) were defined according to the International Classification for Hospital Morbidity Tabulation (ISHMT) taxonomy – ISHMT: 0903 (ICD-9: 410/ICD-10: I21-I22) for acute myocardial infarction and ISHMT: 0908 (ICD-9: 430-434, 436-438/ICD-10: I60-I69) for cerebrovascular diseases. Mortality cases were defined according to the Mortality tabulation list 1 of the ICD-10 (MLT1) – ICD-10: I21-I22 for acute myocardial infarction and MLT1: 1069 (ICD-10: I60-I69) for cerebrovascular diseases. The number of non-fatal cases was calculated by subtracting the number of deaths from the number of all discharges, to avoid double counting of cases [8]. Morbidity and mortality data for the year 2012 (or the year closest to it) within each age group (< 35, 35 – 44, 45 – 54, 55 – 64, 65 – 74, >74) and country were drawn from the European Hospital Morbidity Database [20] and the European Detailed Mortality Database [21], respectively. The average life expectancy in each age group and country for the year 2012 was obtained from the European Health and Life Expectancy Information System [22].

Some countries did not report noise exposure data, as mentioned above, whereas others had missing data on age-stratified morbidity/mortality. Therefore, the analyses excluded some EU-28 countries, and included Iceland, Norway and Switzerland, which had prepared strategic noise maps, although they are not member-states. Overall, 24 European countries were included in our analyses: Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Iceland, Ireland, Italy, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Romania, Spain, Sweden, Switzerland, and the United Kingdom.

2.5. Disability Weights and Duration of Disability

The WHO's burden of disease calculations used the disability weight (DW) for myocardial infarction from the Global Burden of Disease (GBD) 2002 [8, 23]. In our study, we selected a DW for stroke of 0.580 (range: 0.519 – 0.639), which referred to long-term consequences of severe stroke plus cognition problems; it was estimated in a sample of 30 660 people from Europe in 2013 and is representative of European countries [24]. As regards myocardial infarction, the same European study reported DW only for days 3 – 28 following acute myocardial infarction [24], whereas we were interested in days 1 – 2 [8]. Therefore, we used DW = 0.432 (range: 0.288 – 0.579), which is DW for acute myocardial infarction (days 1 – 2) prepared for the GBD 2013 study [25], by combining the GBD 2010 results with those from the 2013 European study [24, 25]. We also checked alternative sources of DWs as sensitivity analysis. (See Table 2) The average duration of disability for myocardial infarction and stroke was assumed to be one year, to calculate losses for each year of life [8].

2.6. Monetary Valuation

As previously done by other authors, the monetary value of one DALY was set at the 2012 GDP per capita in EU-28 [11]. According to Eurostat, it was € 26 500 [26].

Table 2. Alternative disability weights (DW) for myocardial infarction (MI) and stroke and corresponding disability-adjusted life-years lost to road traffic noise in 2012

Source of DW	DW (range)	DALYs (range)
Myocardial infarction:		
GBD 2004 study	0.439 (0.405 – 0.477)	84 883 (84 343 – 85 487)
European 2013 study (acute MI, 3-28 days)	0.098 (0.080 – 0.121)	79 463 (79 177 – 79 828)
GBD 2013 study (acute MI, 3-28 days)	0.074 (0.049 – 0.150)	79 082 (78 684 – 79 574)
Stroke (linear trend for risk):		
GBD 2004 study	0.266 (0.228 – 0.295)	19 002 (18 808 – 19 151)
European 2013 study (moderate stroke)	0.075 (0.059 – 0.093)	18 026 (17 944 – 18 118)
GBD 2013 (moderate stroke + cognition problems)	0.316 (0.206 – 0.437)	19 258 (18 696 – 19 877)
GBD 2013 (severe stroke + cognition problems)	0.588 (0.411 – 0.744)	20 648 (19 744 – 21 446)
Stroke (non-linear trend for risk):		
GBD 2004 study	0.266 (0.228 – 0.295)	186 471 (184 564 – 187 926)
European 2013 study (moderate stroke)	0.075 (0.059 – 0.093)	176 889 (176 086 – 177 792)
GBD 2013 (moderate stroke + cognition problems)	0.316 (0.206 – 0.437)	188 979 (183 461 – 195 049)
GBD 2013 (severe stroke + cognition problems)	0.588 (0.411 – 0.744)	202 624 (193 745 – 210 450)

Note. Sources for DWs – GBD 2004 study: WHO. Global burden of disease 2004 update: Disability weights for diseases and conditions. Available from: http://www.who.int/healthinfo/global_burden_disease/GBD_2004_DisabilityWeights.pdf; European 2013 study: Haagsma JA, Maertens de Noordhout C, Polinder S, Vos T, Havelaar AH, Cassini A, Devleeschauwer B, Kretzschmar ME, Speybroeck N, Salomon JA. Assessing disability weights based on the responses of 30,660 people from four European countries. *Popul Health Metr*. 2015; 13:10.; GBD 2013 study: Salomon JA, Haagsma JA, Davis A, de Noordhout CM, Polinder S, Havelaar AH, Cassini A, Devleeschauwer B, Kretzschmar M, Speybroeck N, Murray CJ, Vos T. Disability weights for the Global Burden of Disease 2013 study. *Lancet Glob Health*. 2015;3(11): e712-23.

3. RESULTS

Table 3 shows the distribution of people exposed to different noise bands and the corresponding relative risks for myocardial infarction and stroke. Noise exposure data were available for a total of 142 069 068 citizens within major agglomerations in the selected 24 countries. More than 60% fell into the reference category < 55 dB considered safe from the cardiovascular effects of road traffic noise. In order not to overestimate the risk of stroke, it was held constant above 70 dB. This was done because the polynomial did not cover exposure > 70 dB. There was a clear discordance between the risks yielded by the linear and non-linear risks, and this discordance was more pronounced in the higher exposure categories, reaching 2% versus 29% above 70 dB.

The source population for our morbidity analyses were 473 648 109 people aged 0 – 85+ from the 24 countries included in the study, which means that the noise exposure data covered about 30% of them. Overall, 1 714 480 discharges and 396 631 ($\approx 23\%$) deaths from cerebrovascular disease occurred in 2012, which yielded 1 317 849 non-fatal cases. There were 876 187 discharges, 236 369 deaths ($\approx 27\%$), and 639 818 non-fatal cases of acute myocardial infarction. Table 4 lists the number of deaths stratified by gender and age group, along with the corresponding life expectancies. Majority of deaths occurred in elderly people (> 65 years).

Table 3. Distribution of inhabitants across different noise categories in the European countries included in the study and corresponding relative risks of myocardial infarction and stroke

Road traffic noise exposure in 2012		Relative risk		
L_{den} [dB(A)]	Number of people exposed (%)	Myocardial infarction	Stroke	
		Linear risk	Linear risk	Non-linear risk
< 55	87 856 368 (62.84)	1.000	1.000	1.000
55 – 59	20 823 700 (14.66)	1.033	1.005	1.028
60 – 64	16 142 100 (11.36)	1.065	1.010	1.084
65 – 69	11 443 300 (8.05)	1.098	1.015	1.172
70 – 74	5 087 100 (3.58)	1.130	1.020	1.294
≥ 75	716 500 (0.50)	1.163	1.020	1.294
Population attributable fraction		0.025	0.004	0.038
Number of non-fatal cases attributed to road traffic noise		15 896	5 112	50 166

Abbreviations: L_{den} – noise level day-evening-night.

Table 4. Distribution of fatal cases of myocardial infarction and stroke and corresponding average life expectancies across sexes and age groups

Age	Men			Women		
	Life expectancy (years)	Number of fatal cases		Life expectancy (years)	Number of fatal cases	
		MI	Stroke		MI	Stroke
0 – 34	60.60	427	586	84.3	126	414
35 – 44	39.00	2 671	1 542	44	501	1 034
45 – 54	29.86	10 203	5 262	34.49	2 037	3 304
55 – 64	21.52	21 878	14 052	25.47	5 790	7 757
65 – 74	14.22	30 632	29 220	17.1	13 200	21 969
> 74	8.00	69 324	112 884	9.6	79 580	198 607
Total		135 135	163 546		101 234	233 085

Abbreviations: MI – myocardial infarction.

Based on the risk estimates and the proportion of people exposed in each noise category, we calculated PAFs for the three disease scenarios. Depending on the trend for risk, 0.4% (5 112) to 3.8% (50 166) of the non-fatal cases of stroke could be attributed to road traffic noise; 2.5% (15 896) of the non-fatal cases of myocardial infarction were road traffic noise-related.

Total YLL due to myocardial infarction were 3 135 782 (1 895 700 for men and 1 240 081 for women), of which 77 905 (47 097 for men and 30 809 for women) were attributed to road traffic noise. Total YLL due to stroke were 4 547 971 (1 873 752 for men and 2 674 219

for women), of which 173 127 (71 328 for men and 101 799) were attributed to road traffic noise, assuming PAF = 3.8% (non-linear risk).

Table 5 shows the final burden of disease estimates. After multiplying the non-fatal cases attributed to road traffic noise by the respective DW and assuming one year as the average duration of disability, the estimated DALYs lost to road traffic noise-attributed myocardial infarction and stroke in 2012 were 84 772 (82 483 – 87 109) and 202 223 (199 163 – 205 183), respectively. Their corresponding monetary value amounted to € 2.25 (2.19 – 2.31) and € 5.36 (5.28 – 5.44) billion, respectively. The linear risk of stroke suggested subtler losses – 20 608 DALYs (20 296 – 20 909) and € 0.55 (0.54 – 0.55) billion. The number of road traffic noise-attributed DALYs we found for ischemic heart disease (12 148 292) and stroke (6 977 256) were 0.70% and 2.90%, respectively, of the total DALYs estimated in the selected countries for the year 2012 [27].

Table 5. Annual burden of disease from road traffic noise-attributed myocardial infarction and stroke in Europe

	Myocardial infarction	Stroke	
	Linear risk	Linear risk	Non-linear risk
Disability weight (range)	0.432 (0.288 – 0.579)	0.580 (0.519 – 0.639)	0.580 (0.519 – 0.639)
YLL	77 905	17 643	173 127
YLD	6 867 (4 578 – 9 204)	2 965 (2 653 – 3 267)	29 096 (26 036 – 32 056)
DALYs	84 772 (82 483 – 87 109)	20 608 (20 296 – 20 909)	202 223 (199 163 – 205 183)
Monetary valuation of DALYs in billion €	2.25 (2.19 – 2.31)	0.55 (0.54 – 0.55)	5.36 (5.28 – 5.44)

Note. YLL – years of life lost; YLD – years lived with disability; DALYs – disability-adjusted life-years.

Sensitivity analyses indicated that the alternative DWs had modest impact on the burden of disease estimates. On the other hand, applying the original third order polynomial of Babisch [14] to estimate the burden of disease from myocardial infarction decreased the PAF to 1.3% and the DALYs lost to 43 686 (42 506 – 44 890). (Data not shown.)

4. DISCUSSION

This chapter extended the WHO methodology to estimate an updated annual burden of myocardial infarction and stroke in 24 European countries. Over 84 000 DALYs and two and € 2.25 billion were lost in 2012 to road traffic noise-attributed myocardial infarction. The burden of stroke varied considerably from about 21 000 DALYs and € 550 million to over 200 000 DALYs and € 5.36 billion, depending on the type of risk used in the calculations.

To our knowledge, this is one of the first burden of disease analyses involving road traffic noise-attributed stroke, as “road traffic noise and stroke” studies are a relatively new research field. Thus, there is no apparent prior example to compare our findings to. On the other hand, comparison with the WHO Global Health Estimates [27] showed that the losses to road traffic noise-attributed stroke in 2012 were almost 3% of the total number of DALYs lost from stroke, which is quite alarming.

Conversely, the burden of road traffic noise-attributed ischemic heart disease was previously assessed by the WHO and amounted to 60 768 DALYs [8]; in other words, it was approximately 20 000 DALYs lower than what we found for myocardial infarction. There are several possible explanations of this discrepancy. First and foremost, we used an updated linear exposure-response relationship between road traffic noise and the risk of myocardial infarction – it was based on eight risk estimates from cohort studies, which made it superior to the old polynomial based on five estimates from both cohort and case-control studies [14]. That polynomial used $L_{den} < 60$ dB as the reference level, meaning that people in this category were not considered to be at risk of myocardial infarction [14]; on the other hand, we used the category < 55 dB. Our linear risk suggested higher risks than the old polynomial below 70 dB (3%, 6.5% and 10% versus 0%, 1.5% and 6.7%). Above 70 dB the case was exactly the opposite (13% and 16% versus 16% and 30%). In spite of this, the risk at lower exposure levels appears to have greater impact on the calculated PAF because only $\approx 4\%$ of all Europeans are exposed to $L_{den} > 70$ dB. As we demonstrated in the sensitivity analyses, using the old polynomial would have underestimated substantially the number of DALYs.

Another noticeable difference with Babisch's analysis is that he did not use raw morbidity/mortality data to estimate the DALYs for IHD in the EU member states, but rather multiplied PAF by the total number of DALYs for ischemic heart disease in high-income European countries [8]. This total number of DALYs was elicited from the GBD 2004 update [28], which likely introduced significant bias, because these DALYs were calculated for the year 2004, while noise exposure data from NOISE was available for 2007 and onwards [12]. Also, Babisch had to assume that "road traffic noise has the similar impact on all ischemic heart disease as on myocardial infarction", because no DALYs were reported for myocardial infarction in the Global burden of disease 2004 report [8]. This means that the number of road traffic noise-attributed DALYs he estimated reflected the burden of ischemic heart disease as a group, but overestimated the burden of myocardial infarction alone. This notion is supported by our sensitivity analysis, which showed that the estimated road traffic noise-attributed DALYs for myocardial infarction were only 0.70% of the total number of DALYs for ischemic heart disease in 2012, and not the anticipated 2.5% (based on the PAF we calculated).

Going further, both the total number of DALYs and the estimated road traffic noise-attributed DALYs in Babisch's study referred only to high-income countries (defined as those having gross national income $\geq$ \$ 10 066 in 2004), whereas the percentage of people exposed to different noise levels was derived from noise maps reported by both high- and middle-income countries [8]. Lower-income countries, however, have poorer road traffic noise control and could have biased upwards the percentage of people in high noise exposure categories. In our study, both noise exposure and morbidity/mortality data referred to the same countries. Also of note, at the time of Babisch's study, the Noise Observation and Information Service for Europe covered agglomerations with $> 250\,000$ inhabitants, while now it reports data on agglomerations with $> 100\,000$ inhabitants as well [8, 12], giving more comprehensive coverage of the population.

A 2013 study commissioned by the Directorate-General for Environment improved the WHO methodology by implemented sophisticated procedures to estimate the burden of traffic noise-attributed hypertension, ischemic heart disease, and stroke [29]. Houthuijs et al. covered the population of 33 European countries (EU-28 plus Iceland, Liechtenstein, Norway, Switzerland, and Turkey), used data from the second round of noise mapping, took into

consideration different sources of environmental noise both within and outside agglomerations, and used raw morbidity and mortality data [29]. They estimated 900 000 additional cases of hypertension in 2012, 43 000 additional annual morbidity, and 10 000 annual mortality cases due to ischemic heart disease and stroke [29]. Road traffic noise within agglomerations was associated with 17 700 hospital discharges, 66 300 YLL, and 6 170 YLD due to ischemic heart disease, and 7 380 discharges, 27 500 YLL, and 3 610 YLD due to stroke [29]. For ischemic heart disease, they used linear RR per 10 dB = 1.05, 95% CI: 1.02 – 1.09), which was based on cohort and case-control studies on both road traffic and aircraft noise and was lower than our risk (RR per 10 dB = 1.065, 95% CI: 1.007, 1.127). For stroke, they conducted an ad-hoc meta-analysis of six road traffic and aircraft noise studies, which yielded RR per 10 dB = 1.04, 95% CI: 1.00 – 1.09. This linearized risk overestimated the linear risk used in our study (RR per 10 dB = 1.010, 95% CI: 0.962 – 1.061) and greatly underestimated the non-linear risk. Despite that Houthuijs et al. covered 33 countries, and we only 24, our burden of disease estimates are greater than theirs, because our risk estimates referred only to road traffic noise and were derived from high quality studies, some of which were not published at the time of Houthuijs et al.'s analysis; we also pooled them under a superior meta-analytical estimator. Further comparison in terms of DALYs and monetary losses is not possible, since these estimates were not generated by the authors [29]. Despite the discrepancies, both our studies clearly indicate that the burden of road traffic noise is higher than previously believed despite the lower percentage of people exposed to $L_{den} > 55$ dB in 2012 in comparison to 2007 [12] and the decreasing cardiovascular disease mortality in many European countries [3]. They also demonstrate that the quality and design of primary epidemiological studies used to generate meta-relative risks has substantial impact on burden of disease estimation.

4.1. Strengths and Limitations

The present study has several limitations. The exposure-response relationship between road traffic noise and myocardial infarction was treated as linear, because its departure from linearity was previously explored and the authors did not find a higher order term to provide significant improvement [18]. Nonetheless, they found that primary studies reporting linear risk showed higher risk of myocardial infarction / ischemic heart disease as opposed to those for which it was derived from categorically reported risks [18]. Therefore, the linear trend we used might underestimate the risk and renders our results conservative, which further reinforces the conclusion that the burden of road traffic noise-related myocardial infarction has increased. Also, from meta-analytical standpoint, it is controversial whether risk estimates for men and women from the same study should be pooled together as if they came from different studies – this creates an issue of statistical dependency. However, this was done, since it is an approach used by Babisch and the WHO with the objective of deriving “a ‘best guess’ pooled relationship for the calculation of population-attributable risks” [8].

As for the risk of stroke, there is not sufficient number of prospective studies published so far to pool a stable cohort risk estimate; therefore, we used both observational and analytical studies. Dzhambov and Dimitrova [19] contended that the exposure-response relationship followed a non-linear risk, but the polynomial they reported was based only on three cohort estimates (two from the same study) and one ecological. Therefore, we also

report burden of disease analyses based on the linear risk they provided. Future meta-analyses should update this pooled risk of stroke with additional cohort studies.

The Noise Observation and Information Service for Europe covered only 30% of the total study population [12], but since it was not possible to obtain exposure data on all settlements, we had to extrapolate these data to the entire population, for which we had morbidity/mortality data. As alleged by the WHO, the impact of this approach is probably minimal, at least in Western Europe, but still could have led to some overestimation of the burden, since traffic in rural and smaller settlements is not as intensive as in major agglomerations [8].

The taxonomy we used to define acute myocardial infarction enables us to interpret the results in terms of myocardial infarction alone, and not ischemic heart disease. Conversely, although we refer to “stroke” throughout the text, the codes we used refer to the more general category of cerebrovascular disease. We extracted morbidity and mortality data on this group of diagnoses, because some of the primary studies pooled by Dzhambov and Dimitrova also defined their outcome as cerebrovascular disease [19]. Thus, although we assumed that the effect of road traffic noise on stroke is the same as on cerebrovascular disease, the burden of stroke may be somewhat overestimated.

CONCLUSION

This study updated the evidence about the burden of disease from road traffic noise in Europe and showed that, despite the reduction in noise exposure above 55 dB, the disability-adjusted life-years due to myocardial infarction are higher than expected. It also reported preliminary evidence regarding stroke, which appears to be associated with considerable socio-economic burden.

DISCLOSURE STATEMENT

We declare no actual or potential conflicts of interests. This study received no external funding.

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Chapter 67

STUDY OF TWO STROKE TECHNIQUES WITH TRANSVERSE MOVEMENT OF THE RIGHT HAND ON CLASSICAL GUITAR USING SURFACE EMG

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ABSTRACT

The purpose of this study is to examine expert playing patterns involving the index and middle fingers of the right hand of two professional classical guitar players using surface electromyographic (EMG) to analyse two different stroke techniques, rest stroke and free stroke, during the performance of a musical composition.

In the area of instrumental technique analysis, there are few studies that use EMG technology. The current study provides an empirical basis to help understand underlying motor control processes in classical guitar performance technique. Further it establishes a process for exploration of instrument technique during music performance.

Two case studies were conducted with professional guitarists who performed the classical guitar piece Estudio No. 5 by Francisco Tárrega (1993). Execution of the piece was recorded using EMG equipment and two video cameras. Subsequent analysis of the fingers involved the synchronisation of EMG data with each of the finger movements made during the performance of technically challenging sections of the music (e.g., transverse movement of the right hand or string crossing).

Results indicated a high degree of stability and consistency in terms of muscular activation, which demonstrated the excellent motor control of the expert guitarists. It is important to note that, each performer showed consistent patterns of motor control for each of the two repetitions of the musical passage even though a comparison of the two performers reveals different surface EMG patterns. These results indicate a measure of

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technique individualization during the performance of the technically challenging section (transverse movement or string crossing) for the fingers (index and middle) and strokes (rest stroke and free stroke).

Keywords: Music performance, instrumental technique, guitar, surface EMG, rest stroke, free stroke

INTRODUCTION

Technical skills are required to perform any musical piece correctly. Expert-level technique serves to free the interpreter from physical constraints in order to allow for greater freedom of musical expression. Thus, technique should be considered a creative element instead of an end unto itself. It is a means of gaining confidence and control, and, through this, magnifying musical effects and improving performance outcomes (Deschaussées, 2002).

Typically, different master teachers have varying approaches to teaching guitar technique, resulting in considerable motor control differences, especially for right hand stroke techniques. Russell (1993) states that although the left hand movements of most advanced guitarists are generally standardised and some diversity exists in terms of different fingerings which may be used to reduce difficulty or for different musical preferences, there are considerable differences in the way the right hand is used. Each performer tends to adopt a personalized posture and technique. However, when discussing technique it is common to refer to the type of positioning of the hands, while what is truly and fundamentally important lies in coordinating multiple seemingly insignificant movements.

Consequently, based on this, successful execution on the guitar can be accomplished in many ways and strings can be plucked in a variety of different manners. Decision-making with regard to the kind of stroke to employ is a primary methodological resource in teaching the instrument. Further, the manner in which a stroke is executed provides the means to develop proper technique and to facilitate the interpretation of musical compositions from different eras and styles. The two methods of plucking the string are: 1) the rest stroke, which involves resting the finger on the neighbouring string after a finger plucks a string, and 2) the free stroke, in which the finger does not rest anywhere, the finger only flexes (De Contreras, 1998; Duncan, 1980; Pujol, 1956; Schneider, 1985).

In general, musical technique and interpretation are founded on teaching methods governed by tradition and pedagogy which are commonly determined by the teacher's own artistic preferences (Visentin and Shan, 2011). In accordance with this, it is noteworthy that current pedagogical methods, which substantively focus on learning technique, are normally based on a series of practical tips which are either generally accepted because they are based on common sense or they have been proposed by teachers of recognised prestige. This reality and the small number of existing empirical studies on guitar technique underscore the relevance of the current study. The results of the study contribute objective information about how to play the guitar and motor control characteristics during performance.

In the field of guitar, one study comparing plucking techniques which did not employ EMG was conducted by Marques, Rosset-Llobet, Fonseca Marques, Gurgel and Augusto (2003). The study indicated rest stroke technique to primarily involve flexion and extension of the proximal phalanges of the three middle fingers. This distinguishes it from the free

stroke technique, which involved incomplete flexion of the middle phalanges of the same digits. One of the conclusions reached by the authors was that rest stroke technique requires greater tension of the flexor muscles, because, after plucking a string, the finger then rests on the neighbouring string.

An additional study of classical guitar plucking techniques (rest stroke and free stroke) conducted by Granda, Barbero and Diaz (2006) examined the degree of muscle involvement using EMG recording for both expert and novice players. The results of the study showed differences in EMG activity between the index and middle fingers of the right hand and significant differences in muscle voltage levels between novices and experts.

In continuation to the work above, Granda, Barbero and Diaz (2007) conducted a study with guitar students at the Professional Music Conservatory of Melilla which determined levels of muscle voltage using surface EMG recording of the right hand. The rest stroke method was confirmed to involve lower levels of muscle voltage, allowing for better development at the beginner and intermediate guitar technique levels, both in terms of skill acquisition and retention.

EMG has also enabled researchers to examine specific techniques used with musical instruments other than the guitar. For example, Bejjani, Ferrara, Xu, Tomaino, Pavlidis, Wu, and Dommerholt (1989) published a research paper which compared three different techniques for playing the piano, confirming the utility of EMG as a means of distinguishing muscle activity for each of the techniques. The results of a study by Moore (1992) on the execution of trills on the piano showed that finger acceleration and EMG activity are presented as phases characteristic of the trill cycle. Furthermore, magnitude and coordination are dependent on speed of the execution of the trill, the dynamic level, and place where the finger hits the key. Another study with professional pianists to complement teaching techniques for piano was conducted by Montes, Bedmar and Sol Martin (1993). The study examined changes in EMG activity by measuring the short abductor muscle of the thumb. The results were used for continued study of the various execution techniques for this instrument. In the same vein, a study examining alternatives to help improve the quality of piano teaching was conducted by Granda, Barbero and Rodriguez (2004). Their results indicated that implementing a program based on information limitation appears to provide significant improvement in the motor control of hand actions, which results in a decreased voltage levels in the thumb extensor muscle and increased values for the little finger extensor, supported by kinaesthetic and proprioceptive information processes.

In terms of bowed string instrument technique, Moore, Hary and Naill (1988) analysed trills executed on the cello to determine the characteristics and speed of finger movement as these relate to the EMG activity of the muscles that control them, providing information for potential biofeedback studies on problems related to instrument practice. Although Fjellman-Wiklund, Grip, Karlsson and Sundelin (2004) used EMG as a measuring instrument to study disorders related to playing stringed instruments, their study showed that EMG can be used to analyse individual differences in the performance of a musical piece and to evaluate different methods of action. And lastly, the aim of the study conducted by Granda, Barbero and Rodriguez (2010) was to analyse and describe the kinematic patterns of the right arm and muscle voltage levels of the anterior deltoid muscles, finger flexors and extensor pollicis of a professional and highly proficient cellist during the interpretation of different sections of a piece of music. Their study demonstrated a stable movement pattern during the performance of a musical piece, and also showed variation in the kinematic values and muscle activity of

the arm segments studied, which were offset by adjustments in other segments of the arm. Another important finding of the study was the fact that the driving force for the right arm in a cellist's technique was the shoulder. The hand and fingers act to correct and absorb shock from the general movements stemming from the shoulder.

Using the abovementioned research as a foundation, the current research aims to determine muscle voltage levels through surface EMG recording during the performance of a musical piece by two highly proficient professional guitarists for two different stroke techniques in order to describe and analyse motor control patterns used in guitar performance which are the result of attaining expertise in the execution of motor skills for both plucking methods.

METHOD

This study took the form of a case study experimental design with a single post-test. Each participant was considered a unique case, since the aim was to gain insight into the participants' performance behaviours (the patterns of activity of each participant) and to investigate possible intra-subject differences.

In the first phase of the experiment, an expert with over 40 years of experience as a teacher and more than 25 years dedicated to musical interpretation was asked what classical guitar piece would be most suitable for the study.

By this process, *Estudio No. 5* by Francisco Tárrega (1993) was selected for the test protocol. The participants were then asked if they agreed that the piece would be useful for studying both right hand plucking techniques (rest stroke and free stroke) using the index and middle fingers.

This was necessary because, for many compositions, there can be a variety left and right hand fingering and plucking patterns. The participants agreed as to the appropriateness of the selected composition.

During this phase, two guitar virtuosos (experienced professionals) were asked to practice the aforementioned *Estudio* using both stroke techniques (rest stroke and free stroke) for 16 minutes a day (8 minutes of rest stroke practice, and 8 minutes of free-stroke practice) over the period of one month. They were also asked to take extreme care to keep their fingernails in optimum condition during their practice so that they would be in excellent condition on the day when the data was to be recorded.

In the second phase, data were collected on the levels of muscle activity and finger actions. Prior to starting the experiment, all participants performed warm-up exercises, basically consisting of scales and passages from the *Estudio*. They were told to take as much time as they needed to warm up, to ensure they were fully prepared to perform the test piece. In the data recording session, each guitarist played the *Estudio* 4 times: 2 times with the rest stroke technique and 2 times with the free stroke technique, starting with the technique each preferred (see Figure 1).

	Repetition 1	Repetition 2	Repetition 3	Repetition 4
Interpreter 1	Free stroke	Rest stroke	Free stroke	Rest stroke
Interpreter 2	Rest stroke	Free stroke	Rest stroke	Free stroke

Figure 1. Order in which each of the guitarists interpreted the piece.

The guitarists interpreted the piece from memory in each of the four repetitions, and in each repetition the guitarists employed the same fingerings for the left and right hands. All of the soloists' performances were recorded using EMG equipment and two digital cameras. Surface EMG measurements were taken using the I-330 12-channel recording system (4 EMG), (J & J Engineering). Bipolar EMG recordings of extrinsic finger flexors were obtained using pairs of Ag-AgCl surface electrodes. EMG signals from the flexors of each finger were recorded on an ASUS F3J model laptop. Two Casio EXF1 digital cameras recording at 100 frames per second were used to record video and audio of the interpretation. These were positioned to facilitate subsequent analysis of the fingers involved in the study, the index and middle fingers, and for synchronisation of EMG data with each one of the moments (actions) of the fingers during the performance.

For surface EMG recording, electrodes were placed in accordance with the recommendations of Basmajian and Blumenstein (1989). Two pairs of electrodes were placed parallelly and longitudinally from the proximal to the distal insertion, so that each pair recorded tension generated by the actions of each of the fingers studied, with each compartment separated by 13 mm, the distance recommended by Shan and Visentin (2005) to avoid the cross-talk effect between the segments of muscle studied. This placement of surface electrodes did not allow the activity of the FDS finger superficial flexor to be separated from the FDP finger deep flexor. However, as force was applied in the distal phalanges of the finger position studied the deep flexor FDP was the main contributor to finger forces (Li, Zatsiorsky and Latash, 2000). The reference ground electrode was placed on the dorsal side of the forearm. All the EMG signals were sampled at 100 hertz, amplified, and then filtered using a high-pass filter at 10 hertz and a low-pass filter at 500 hertz, effectively resulting in band pass data of 10-500 hertz.

Each pair of electrodes recorded voltage generated by the muscles needed to produce the actions of each of the fingers studied. This allowed the researchers to verify the relationship between finger placement and muscle activity in order to analyse the motor control patterns of the finger segments. To ensure the validity and reliability of data recording, the activity of each finger was sampled separately with EMG recording software. The level of activation of performing fingers was verified, while the fingers which remained inactive showed minimum and unchanged tension.

For data analysis, a section of the Estudio was selected (Figure 2). In this passage, performers only use the index and middle fingers of the right hand. This section presented a series of relevant guitar technique challenges to be used in subsequent study of the action of the fingers which are the primary object of this study. The results for the technically

challenging section selected correspond to transverse displacement of the fingers of the right hand, i.e., string crossing.



Figure 2. Selected passage (measures 3 to 7) of Estudio No. 5 by Francisco Tárrega (1993).

For the analysis of surface EMG measurements, the data collected was first synchronised with the videos recorded of the participants' performance using EMG recording software. Post-synchronisation, the videos were viewed to identify precisely which situations corresponded to the musical passages that were the focus of the study. Using this process, sections of EMG data that matched these video segments were then identified.

After extracting the audio-video recordings and the EMG for the identified musical passages, each section was viewed frame by frame in order to precisely and rigorously determine the EMG value for each moment of finger control during the execution of the stroke, providing the information that would later be used for analysis and interpretation of emergent behaviours in each situation analysed.

RESULTS AND DISCUSSION

Surface EMG patterns for the index and middle fingers and the technique employed (rest stroke or free stroke) in each case are indicated below for some of the musical segments analysed. In each instance, the guitarists were performing a transverse hand movement, i.e., moving the fingers from one string to another laterally across the fret board. Transverse right hand movement requires correct left hand finger posture and therefore presents a technical challenge which is practiced from the initial stages of studying the instrument (Carlevaro, 1979).

Participant 1

As illustrated in Figure 3, during the execution of identified technical passage using the rest stroke technique, the guitarist (participant 1) changed from the first string plucked with the middle finger to the second which was plucked with the index finger. When the second string is plucked, a minimal rise in voltage is observed which decreases as the index finger action ends and comes to rest on the neighbouring string. The higher voltage levels attained in the action of the plucking finger is the result of the intensity with which the guitarist executes this moment of the interpretation, in accordance with the requirements of the piece. In this same vein, in a study of pianists, Montes et al. (1993) noted that variation in EMG activity was related to stroke intensity.

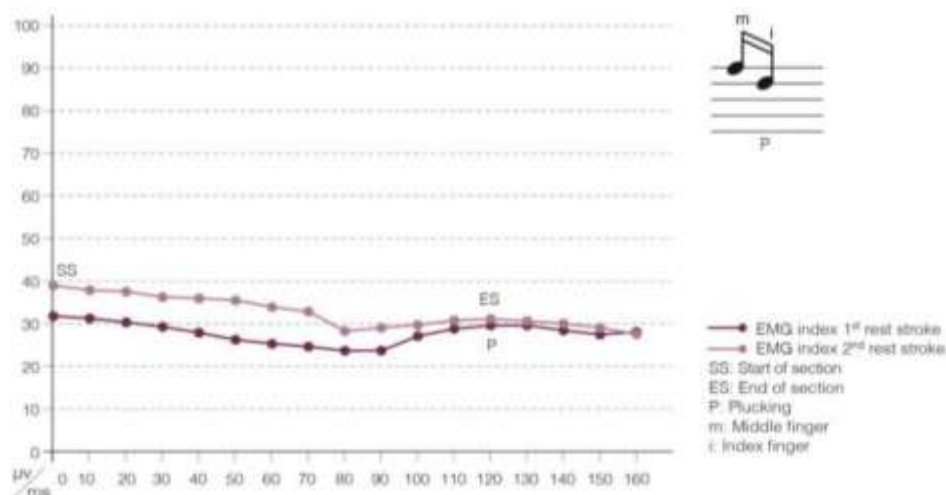


Figure 3. Surface EMG pattern for the index finger of the right hand, rest stroke, measure 5, second part.

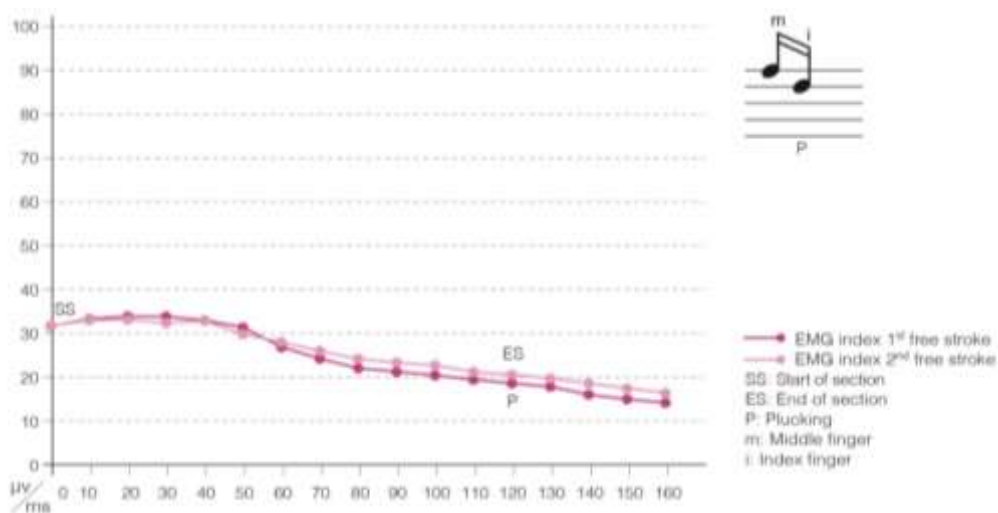


Figure 4. Surface EMG pattern for the index finger of the right hand, free stroke, measure 5, second part.

In Figure 4, while crossing strings using the free stroke technique, the explanation for the surge in muscular voltage levels at the beginning of the action when approaching the string, and the reduction of these in the final moments of the action, may lie in the fact that when the free stroke is executed the guitarist does not have a point of support or reference to complete the action, and must apply greater control over the movement and speed of execution prior to plucking the string. To do otherwise an uncontrolled movement may occur in the impulse of the final action, and, as a consequence, an unintended musical effect may result.

This data corroborates the findings of Carlevaro (1979) on the mechanics of attack, which indicates that work is not completed with the attack itself, but must be complemented by counterforce as strong as or stronger than the original action to avoid abandoning the finger once plucking is completed. This is due to the fact that, in this musical situation, results

indicate that the force applied is greater in the phase of impulse towards the string and, at the time of completion of the impulse, this may require compensatory action of the extensor muscles of the fingers to balance the action. This counteraction demonstrates the system may dictate different activation thresholds for each of the muscle groups in order to achieve greater control over the action (Feldman, 1998).

In Figure 5, during the execution of the technically challenging section using the rest stroke technique, the second string was plucked with the index finger, then the first string was plucked with the middle finger. At the moment of plucking, the lowest muscle voltage value was recorded in both repetitions.

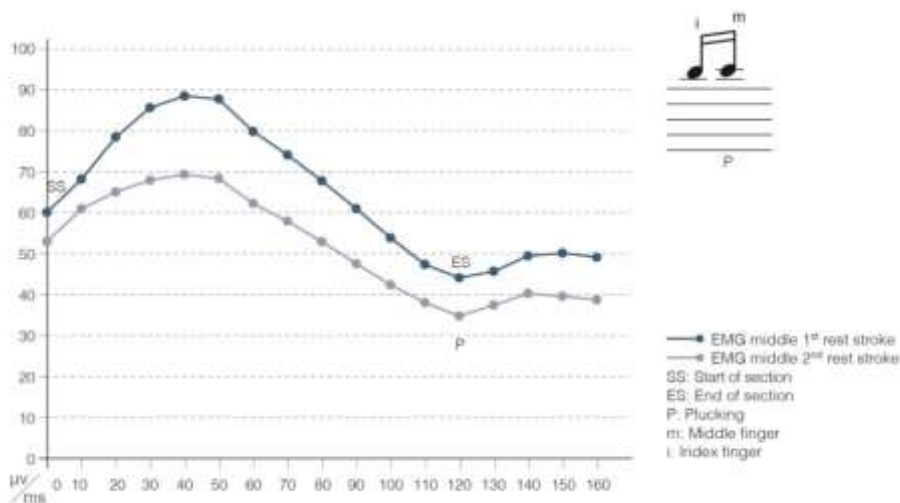


Figure 5. Surface EMG pattern for the middle finger of the right hand, rest stroke, measure 3, second part.

The explanation for why voltage levels increase at the beginning of the action of the middle finger may be that at this moment an impulse must be generated to ensure that the finger reaches the corresponding string and presses it with the required force while counteracting the action of the finger extensors in the opposite direction.

As illustrated in Figure 6, when the free stroke was, the level of activation (voltage) dropped at the moment of plucking and increased at the end of the action, i.e., when the middle finger had finished plucking.

The reason why the maximum level activation (voltage) is reached when the string has been plucked (graph value 17), is due to adjustments that may be related to a choice of musical interpretation - something the interpreter decided to apply when performing an ascending scale.

Lastly, the temporal stability presented by Participant 1 in all of the musical passages analysed using both fingers and techniques should be noted.

This provides a clear example of the degree of precision and control that expert performers employ when interpreting a piece.

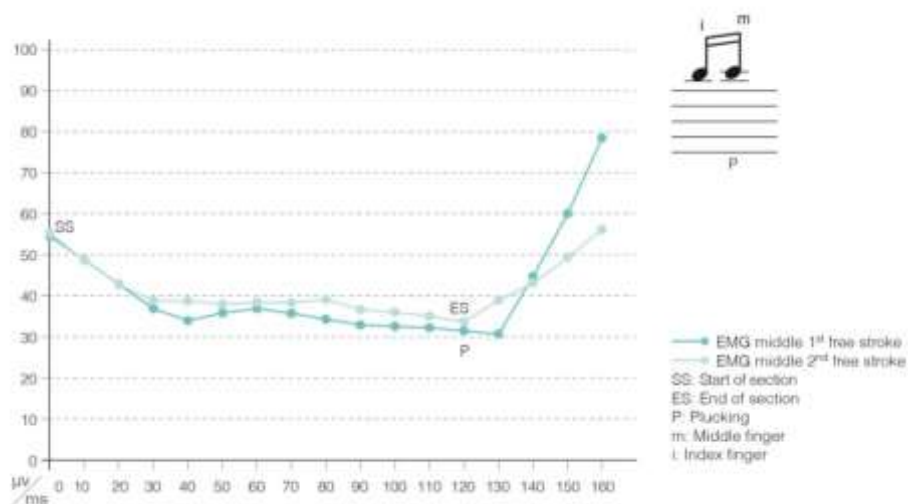


Figure 6. Surface EMG pattern for the middle finger of the right hand, free stroke, measure 3, second part.

Participant 2

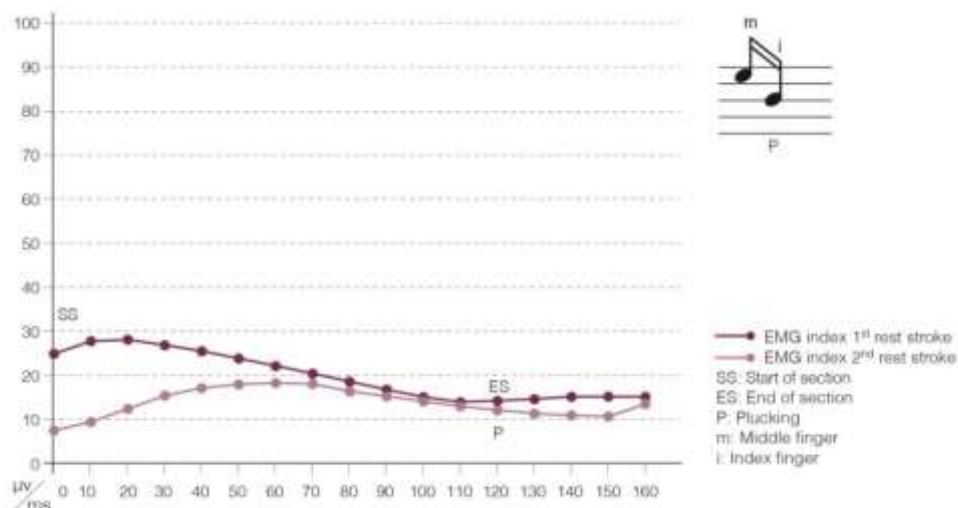


Figure 7. Surface EMG pattern for the index finger of the right hand, rest stroke, measure 6, first part.

In Figure 7, during the execution of the technical section using the rest stroke technique, the participant (guitarist 2) changes from the first string plucked with the middle finger to the second which is plucked with the index finger.

This activation pattern, which lasts from the start of the action until the time of the stroke, can be explained by the need to adjust finger action to control intensity in the execution of these notes.

In Figure 8, when the guitarist plays the passage using the rest stroke technique, the decrease in muscle voltage as the finger approaches the strings to be plucked and during plucking may be motivated by the desire to ensure greater motor control over the execution of the plucking, and to properly adjust this to the intensity with which the corresponding note is to be executed.

As shown in Figure 9, when the guitarist changed from plucking the second string with the index finger to plucking the third string with the middle finger, an increase in the level of muscular voltage was registered during the stroke. This may be due to an attempt to produce a specifically desired tone.

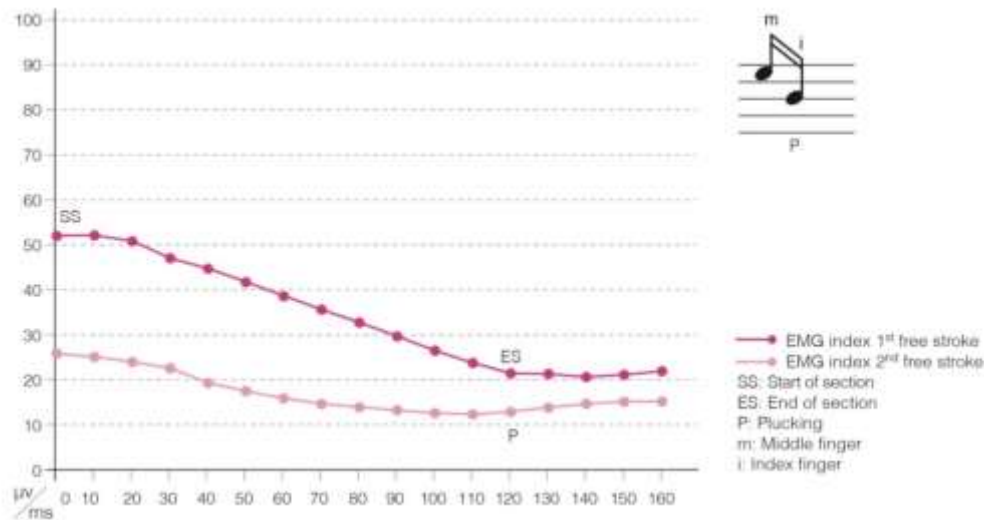


Figure 8. Surface EMG pattern for the index finger of the right hand, free stroke, measure 6, first part.

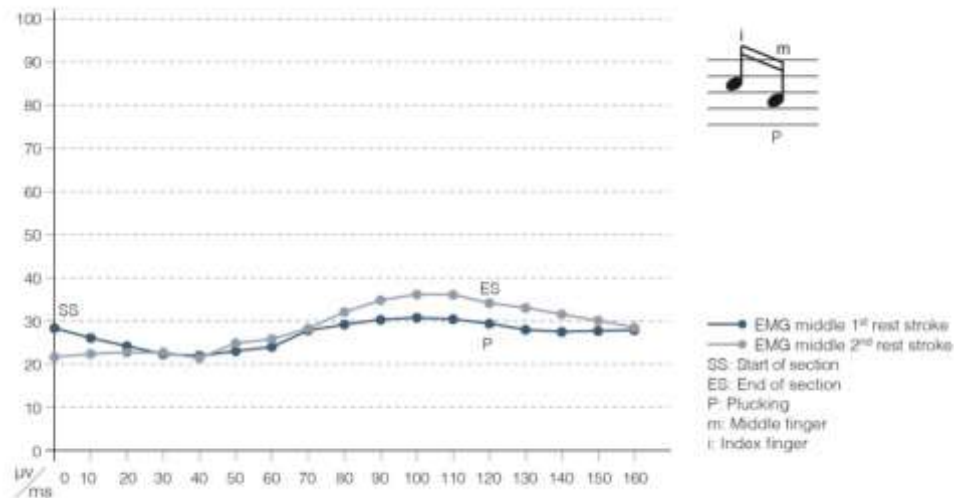


Figure 9. Surface EMG pattern for the middle finger of the right hand, rest stroke, measure 6, second part.

Also, when changing the fingering of the right hand required for the interpretation of the score (index and middle fingers), a situation arises in which the fingering is reversed (with the index finger on the second string and the middle finger on the first string). It is important to note that when playing guitar scales, the index and middle fingers are used primarily, but the ring finger and/or thumb are also used to avoid awkward string crossings Wen-Tzu (1999). The graph shows that this issue does not affect the EMG voltage pattern. The levels remain constant in the two repetitions in the most important moments, which correspond to contact with the string, plucking, and the end of the action (graph values 9-17).

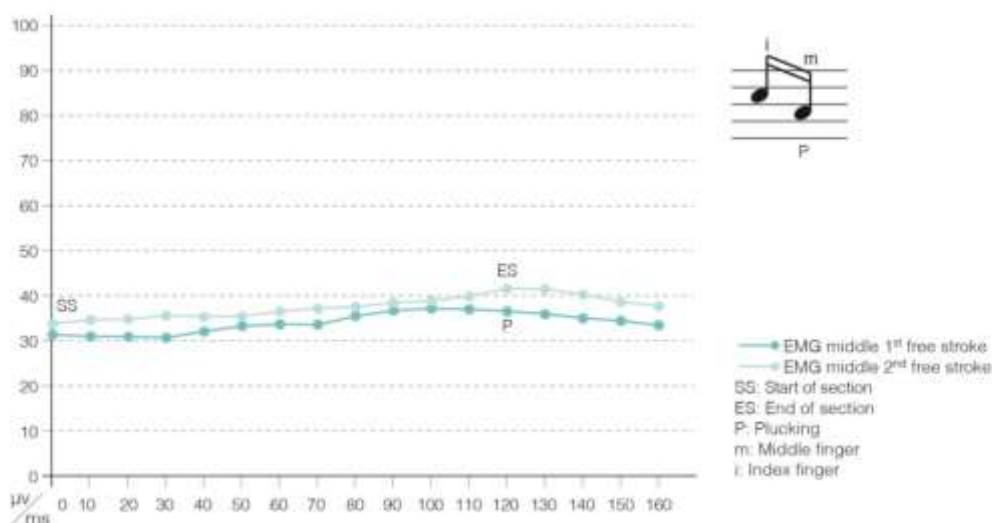


Figure 10. Surface EMG pattern for the middle finger of the right hand, free stroke, measure 6, second part.

In Figure 10 (as in Figure 9 with the rest stroke technique), when the guitarist performed the musical passage using the rest stroke technique, the highest values were recorded at the moment of the stroke. This is comparable to other situations using the index finger in both techniques mentioned above. This may be due to anatomical-functional limitations which intervene in the action of the middle finger, i.e., the middle finger is not completely independent like the index finger because when it is free next to the index finger the intertendinous connection joins it with the ring finger (Latarjet and Ruiz Liard, 1990). These higher values may also be the result of adjustments made by the performer meant to facilitate the action of the finger. In this situation, the same reverse fingering mentioned above takes place. This added challenge did not affect the surface EMG pattern.

Lastly, Interpreter 2 presented similar temporal parameters in all of the repetitions and situations analysed, demonstrating a clear mastery of musical execution.

CONCLUSION

The results of this study indicated a high degree of stability and consistency in terms of muscle requirements for both participants, which is evidence of the excellent motor control

required to become an expert guitarist. It is important to note that each of the performers presents different surface EMG patterns in the fingers (index and middle) and for the stroke systems studied (rest stroke and free stroke), but inter-subject trials were similar for all of the musical passages.

The data showed that Participant 1 presented the same index finger pattern at the moment of the stroke and afterwards in both techniques. It also showed an identical pattern for the middle finger when approaching the strings, during the attack, and in the final stages of the actions.

Participant 2 presented a single pattern for the index finger and a different pattern for the middle finger on the approach to the strings, during the stroke, and at the end of the action.

Of particular importance is the fact that sometimes the participants presented variable muscle activation values in the initial and end phases of their actions for each of the repetitions studied in all musical situations, but there was clear pattern of similar values – which at times were even identical – in key moments of technical difficulty such as contacting and plucking the strings. This means that muscle activation during plucking is directly related to the intensity of execution that the guitarists chose to employ for the interpretation of each note.

Lastly, it should be noted that both performers maintained a high degree of temporal stability in the different musical passages, which demonstrates the extremely high degree of accuracy applied to the interpretation of the musical piece.

In summary, the current study provides an empirical basis to help understand underlying motor control processes in classical guitar performance technique. Further it establishes a process for exploring instrumental performance technique that may be understood as arising from performers' individualized musical motivations or approaches to performing on the guitar.

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Chapter 68

NYSTAGMUS IN POSTERIOR FOSSA STROKE PATIENTS

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INTRODUCTION

In this chapter we will describe briefly the pathophysiological mechanisms of central nystagmus generation, proceed with the description of bedside examination of the patient with sudden onset of vertigo and unsteadiness. We will present the results of 9 patients with stroke diagnosis who were during the year 2013 admitted to our Neurological Emergency Department and who came with sudden onset of vertigo and unsteadiness as the leading symptom of illness. On the basis of this results we will discuss the importance of recognition of the impairment of central vestibular pathways as solely symptoms or additional symptoms in the early diagnosis of cerebral stroke. The therapeutic guidelines will be presented as well.

Pathophysiological Mechanisms of Central Nystagmus Generation.

Vestibular pathways run from vestibular nerve and vestibular nuclei mostly through the fibers of medial longitudinal fasciculus (MLF) to the oculomotor nucleus and supranuclear integration centers in the midbrain (interstitial nucleus of Cajal (iC) and rostral interstitial medial longitudinal fasciculus (riMLF) nucleus).

Sometimes pathophysiology of the anatomical structures can explain the visible nystagmus but occasionally the topography of the nystagmus generation is not so simple.

Nevertheless, we will try to present the pathophysiological mechanisms which lead to the generation of central vestibular nystagmus.

Gaze Holding

To start an eye movement, a burst of activity from motoneurons is needed. To maintain the gaze in one position, activity from neurons which differ cytoarchitecturally from motoneurons is necessary. Those neurons provide a constant tonic input to the ocular muscles and they are involved in gaze-holding. They form the so called neural integrator and additional clusters of cells which are interspread between and around the fasciculus of MLF [1]. The neural integrator for horizontal eye movements is located in the vestibular nucleus/nucleus prepositus hypoglossi complex [2,3]. Cell clusters around the crossing of the MLF fibers in lower pons (paramedial pontine reticular formation (PPRF)) are demonstrated to be involved in gaze holding as well [4]. In addition, the flocculus of the cerebellum supports gaze holding. For vertical gaze holding the (iC) in the mesencephalon is proved to be responsible [5].

If maintenance of stable conjugate eye deviation away from the primary position is not possible, the eyes drift back to the center and a corrective saccade (or fast phase) brings the eye back to the desired position. This happens for as long as the attempt of holding the eyes fixed on one object in the lateral position is present. The result is gaze-evoked nystagmus. The gaze evoked nystagmus changes it's direction with the change of gaze position. It is always present in the direction of gaze (Figure 1). The gaze evoked nystagmus generally appears on attempted gaze, but it can also occur spontaneously because of the difference between the null and the midposition of the eye. Internuclear ophthalmoplegia (INO) is a pathological oculomotor sign attributed to unilateral or bilateral MLF lesions. It is characterized by slowing of adduction of the eye on the side of the lesion and a gaze evoked nystagmus of the contralateral eye. INO can be present bilaterally [6]. The slowing of adduction is better visible in cases of larger compared to smaller lesions.

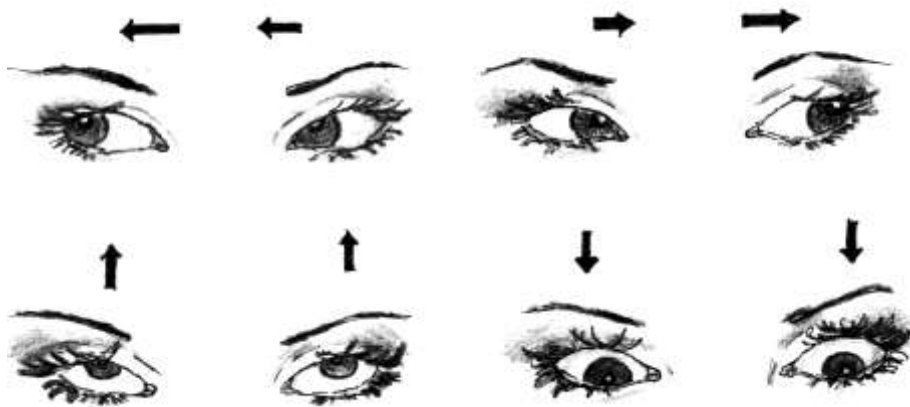


Figure 1. Gaze evoked nystagmus to the right, to the left, upwards and downwards. Arrows show the direction of eye movements and the amplitude of the movement of each eye.

Yaw, Roll and Pitch Plane

According to Brandt and Dieterich [7] signs and symptoms of vestibular dysfunction can be divided according to three planes of action of the vestibuloocular reflex (VOR): yaw, roll and pitch plane.

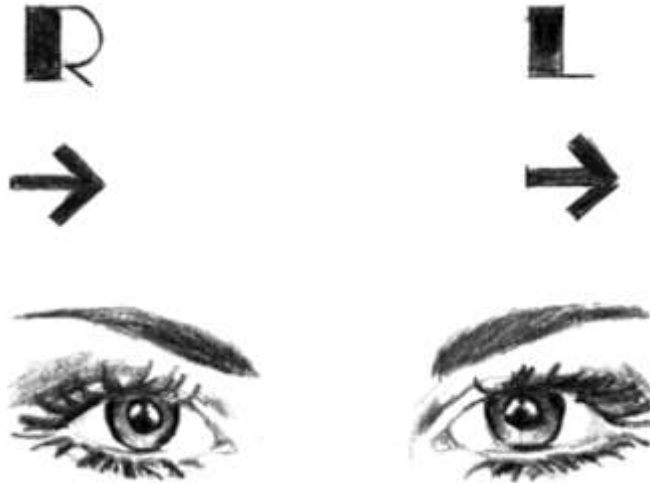


Figure 2. Horizontal nystagmus to the left, the arrows show that both eyes are moving with the same amplitude and in the same direction.

Spontaneous horizontal nystagmus is a VOR dysfunction in yaw plane (Figure 2). In most cases it is caused by the lesion of vestibular sense organ or nerve. It is rarely caused by central lesions but it can be present if the lesion involves a small part of the medial and superior vestibular nuclei and the adjacent centers for gaze holding (PPRF). Postural signs in the same plane are lateral head tilt and lateral body tilt toward the side of the lesion.

Roll Plane

If a unilateral central lesion of the vestibular afferent pathways happens, it mostly affects the so called graviceptive pathways which transduce the information from vertical semicircular canals and otoliths. The result is an imbalance of the vestibular input (tone imbalance) in the roll plane with a torsional nystagmus (Figure 3).

Lesions of iC cause an ipsiversional torsional nystagmus while lesion of riMLF nucleus is responsible for a contralateral torsional nystagmus [8]. Postural signs are head and body tilt to the side opposite to the lesion, sometimes skew deviation with the undermost eye on the side opposite to the lesion and ocular torsion (Figure 4). Skew deviation can also accompany peripheral vestibular lesion of utricle [9]. Ocular torsion is present, but it can not be seen by bedside examination. It can be demonstrated by fundus photography. Therefore we will not discuss this symptom further. The triad of symptoms head tilt, skew deviation and ocular torsion is called ocular tilt reaction (OTR). It can be present some peripheral as well as central lesions of the vestibular pathways up to the mesencephalon.

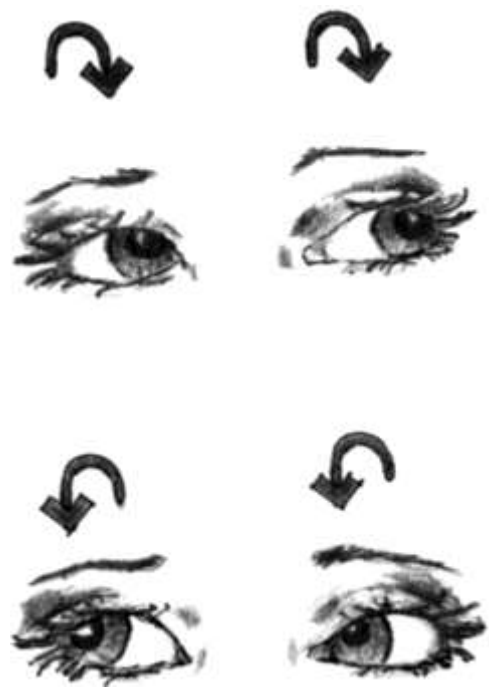


Figure 3. Torsional nystagmus. The arrows show the direction of eye movements.

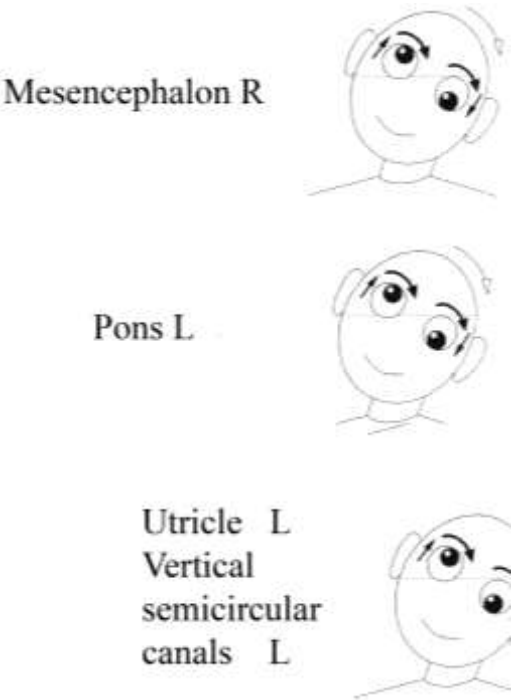


Figure 4. A schematic drawing of the vestibular syndromes in roll plane. On the labyrinthine and pontine level, head tilt and skew deviation are toward the lesion side, on the mezencephalic level they are toward the contralateral side (according to Brandt and Dieterich 1995).

Pitch Plane

Bilateral lesions of the central vestibular pathways result in vertical upbit nystagmus (pitch plane) and forward or backward body tilt.

Flocculus

Flocculus of the cerebellum supports the gaze holding, unilateral lesions of flocculus and its connections to the vestibular nuclei result in gaze holding deficit (Buettner and Grundei 1994) and failure of fixation suppression of the vestibular nystagmus [10]. Bilateral lesions of flocculus result in downbeat nystagmus.

BEDSIDE EXAMINATION

Only neurootological tests which can enlarge the examination done by neurologist, and only the tests which can be done on bedside will be discussed. Various very useful neurootological tests for which some instruments are needed, will be skipped. The performance of tests already described in details previously in this book will not be repeated.

The spontaneous nystagmus caused by a damage or irritation of the vestibular apparatus/nerve was already previously described. If the eye movements are horizontal, our attendance must be directed to both eyes because if both eyes are moving with the same amplitude and in the same direction, we can call it horizontal “vestibular” nystagmus (figure No 2). If both eyes a moving spontaneously with torsion of both eyes in one direction, it is the spontaneous torsional nystagmus (figure No 3). If the eyes are moving spontaneously up or spontaneously down, this is the spontaneous vertical nystagmus.

If the eyes are not moving spontaneously at the moment of examination, we provoke the nystagmus by positional tests. The horizontal vestibular nystagmus can be provoked also if the patient fixate on a target 30° to the right and then to the left. The nystagmus will occur only in one direction and both eyes will still be moving with the same amplitude (according to Alexander’s I degree).

The next test used to provoke a horizontal “vestibular” nystagmus is the head shaking test. Nystagmus duration depends on the magnitude of the vestibular damage.

Next we try to provoke the gaze evoked nystagmus. The patient fixate on a target 30° to the left, to the right, up and down. The nystagmus which appears on gaze fixation is called the gaze evoked nystagmus. Usually with horizontal gaze fixation the amplitude of the abducted eye is larger then the amplitude of the adducted eye (figure No 2). The nystagmus changes it’s direction with the direction of gaze. If the patient moves his abducted eye about 40° laterally the so called end point nystagmus can occur which is not a sign of illness. This nystagmus usually stops after a few second. Asking the patient to fixate his gaze at this position, we can sometimes discover a gaze evoked nystagmus which is a remnant of a previous illness of the peripheral or central vestibular pathways.

The gaze evoked nystagmus can be caused by the damage of flocculo-vestibular connections. It changes the direction with the direction of gaze. According to our personal observations, in this case the evoked nystagmus is with equal amplitudes on both eyes,

usually with low frequency. For this observation we don't have any experimental confirmation.

Vertical fixational nystagmus appears at upward or downward gaze fixation. More often is the upward nystagmus.

Skew Deviation

By horizontal head impuls (or head thrust) test a recent horizontal canal paresis can be demonstrated. The observer can be uncertain about the result in the case of mild or moderate lateral canal paresis.

Caloric test is the other way to test if canal paresis exists. The standard bithermal caloric test with hot (44°) and cold (30°) water can not be easily performed at bedside. Therefore we use the test described by Torok. The test is done first with a weak stimulus (10 ml) and then with a strong stimulus (100 ml) of 20° cold water. The postcaloric nystagmus duration is measured in seconds. The postcaloric nystagmus lasts for about 50 seconds after weak stimulus application, and about 90 seconds after the strong stimulus application. If a more than 25% asymmetry between the left and the right side exists, the patient suffers of a horizontal canal paresis [11]. The general recommendation for the time of caloric test accomplishment is more than 4 days after beginning of sudden vertigo.

The Vestibulospinal Tests

The past pointing test is positive in terms of deviation of the arm to one side during the attempt to touch the examiners finger with closed eyes. It is a nice demonstration of hypotonia of arm muscles after sudden impairment of the vestibular apparatus.

Romberg test and "sharpened" Romberg test although not specific for vestibular instability, can be used at the beginning of the illness when a clear inclination or swaying to one side can be demonstrated. During the "sharpened" Romberg test the patient stand in the tandem heel-to-toe position with eyes closed and arms folded against the chest. He inclines or falls toward the impaired side.

Tandem walking (heel- to- toe) is not possible in acute vestibular illness.

METHODS

We performed a retrospective study of 723 in-patients of our Neurological Emergency Department admitted to the Department during the year 2013. There were 53% of women, 47% of men, between 18 and 78 years (mean 42 years). The patients with sudden vertigo and sudden instability as the dominant symptom of illness were selected. Patients with vertigo and hearing loss were excluded from this study. All patients where first seen by a neurologist. A computerized tomography (CT) scan was performed before the admission and repeated within 22 to 36 hours, or earlier, in cases of clinical deterioration. In some patients a diffusion weighted magnetic resonance imaging was performed (MR) as additional neuroimaging tool.

Each patient with vertigo was also seen by a ENT specialist-neurotologist, on the first or on the second day of admission.

In all patients with suspected stroke, the evaluation of blood vessels of head and neck was done by using Carotid Doppler Sonography (CDS), Transcranial Doppler (TCD) and CT angiography.

Therapeutic process for patients with suspected stroke included their treatment in the stroke unit following the protocol which was made based on recommendation of current guidelines [12, 13].

RESULTS

Out of 723 in-patients, only 22 were hospitalized because of sudden vertigo and sudden instability as the dominant symptom of illness. After precise clinical examination, 9 patients were diagnosed as cerebral stroke, 13 as vestibular neuronitis.

The results of the patients with stroke are shown on Table 1.

Table 1.

Name	Years	Imaging	Diagnosis	Additional neurological signs	Neurotological signs
M.S.	54	MR and CT	Cerbl inf R Sten vert a R	Yes	spont H ny to the L HIT: paresis R cal test: paresis R FFS R side vest spin tests: inclination to the R
M.M.	75	CT	Pontin inf L and cerbl inf R	Yes	paresis n. VI L gaze evok ny to R HIT: uncertain cal test: symmet vest spin tests: without incl to one side
K.S.	54	CT	Cerbl inf R Ocl vert a R	Yes	gaze evok ny to the R HIT: symmet cal test: symmet FFS on the R vest spin tests: ataxia
V.M.	73	CT	Cerbl inf L	No	gaze evok ny to the R and to the L HIT: symmet cal test: symetr with FFS bil vest spin tests: ataxia
V.D.	45	MR	Mezenceph inf L	Yes	Spont torsional ny to the R Skew dev with the lower R eye HIT: uncertain cal test: symmet vest. spin tests: incl. to R
D.B.	53	MR	Thalam inf L Sy trunci cerebri	Yes	Paresis n. VI L gaze evok ny to the R HIT: symmet cal test:symmet vest. spin tests: incl L
M.M.	61	MR	Pontine inf R Ocl vert a R	Yes	INO R HIT: uncertain cal test: not performed vest spin tests: not performed
N.J.	26	MR	Mezencephalic inf L	No	Spont torsional to the R Haed tilt R HIT: uncertain cal test: symmet vest. spin tests: incl to the R
K.S.	55	CT	Cerbl inf R Sten vert a R	Yes	Gaze evok ny to R HIT: symmet vest. spin tests: ataxia

Cerbl - cerebellar, inf - infarction, R - right, sten - stenosis, vert -vertebral, a - artery, L - left, ocl - occlusion, mezenceph - mezencephalic, Thalam - Thalamic, Sy - syndroma, Midbr - Midbrain, spont - spontaneous, H - horizontal, ny - nystagmus, HIT - head impuls test, cal - caloric, FFS - failure of fixation suppression, vest spin – vestibulospinal, gaze evok – gaze evoked, symmet – symmetrical, incl – inclination, dev – deviation.

The patients were between 26 and 75 years old, 5 female, 4 male. CT scan was done in all of them at the moment of admission, and was repeated in following 22-36 hours from the symptom onset. MR was done in 5 patients.

Isolated cerebellar infarction was present in 4 patients, pontine and cerebellar infarction in 1 patient. Isolated pontine infarction in 1, mezencephalic infarction in two patients, thalamic infarction together with brainstem syndrome in one.

Unilateral stenosis or occlusion of vertebral artery was demonstrated in four patients (3 with cerebellar infarction, 1 with pontine infarction). Neurotological finding is listed in details. Except neurotological positive finding, additional neurological signs (involvement of long pathways, other cranial nerve (except vestib. nerve and abducens nerve) lesions, hemihypesthesia, dismetria) were present in 7 patients, in two they were not.

Spontaneous horizontal nystagmus was present in one patient, peripheral vestibular etiology was demonstrated by head impulse test (HIT) and caloric testing, as well as by vestibulospinal tests (toward one side). The cerebellar involvement was demonstrated with unilateral failure of fixation suppression (FFS).

Spontaneous torsional nystagmus was present in two patients. One had torsional nystagmus to the left side, skew deviation with the lower right eye, inclination to the right while performing the vestibulospinal tests, symmetrical HIT and symmetrical caloric test. The other had spontaneous torsional nystagmus to the right, head tilt to the left and inclination to the left while performing the vestibulospinal tests. HIT was symmetrical as well as the caloric test.

Three patients with cerebellar infarction had gaze evoked nystagmus: one bilateral while two to the side of cerebellar lesion. HIT and the caloric test were symmetrical in all three patients. One had bilateral FFS, the other two unilateral, on the side of cerebellar lesion. Ataxia was present in all patients while performing the vestibulospinal tests.

The patient with pontine and cerebellar lesion had abducens nerve paresis on the left side and gaze evoked nystagmus to the right. HIT was uncertain, caloric test symmetrical, and he did not incline to one side while performing the vestibulospinal tests.

One patient had isolated pontine lesion on the right side. Internuclear ophthalmoplegia (INO) was present at the side of lesion. HIT was judged as uncertain. Caloric test and vestibulospinal tests were not performed because patient's general condition worsened.

In one patient a thalamic infarction was visible with MR, but clinical examination demonstrated that a brainstem syndrome is present in addition. An abducens nerve paresis was present on the left side, a gaze evoked nystagmus on the right side. HIT was symmetrical, caloric test symmetrical, during the vestibulospinal tests he inclined to the left.

Therapy

Only 1 of 9 patients arrived at the hospital within the therapeutic window of 4.5 hours for administration of intravenous thrombolytic therapy and was consequently treated with it. The secondary stroke prevention was done by using Aspirin, dose 300 mg, which was introduced in the first 48 hours from symptoms onset. Patients were also treated with intravenous solution (0.9% Sodium Chloride and Ringer) as well as antiemetic, and other therapy for control of cerebrovascular risk factors.

DISCUSSION

To recognize the impairment of central vestibular pathways in patient with sudden onset of vertigo and unsteadiness is of crucial importance in neurological practice, especially in Neurological Emergency Units.

During last few years the importance of this recognition was stressed in several publications (14, 15, 16, 17).

Among 723 patients hospitalized at Emergency Neurology Department of Clinical Centre of Serbia, 22 came with sudden vertigo and unsteadiness as a leading symptom of their illness.

Thirteen of them were diagnosed as vestibular neuritis and treated accordingly. Nine of them were diagnosed as cerebral stroke. We analyzed which neurotological signs accompanied which localization of stroke and how effective single diagnostic procedures were in establishing the diagnosis.

In cerebellar stroke patients, only one patient had a peripheral vestibular impairment additionally. This impairment was recognized by typical signs of peripheral vestibular damage: horizontal nystagmus, positive HIT, body inclination toward the lesion side, unilateral paresis in caloric test. The cerebellar sign was in this case the unilateral FFS. Besides neurotological, the patient had neurological signs as explained on the table. The neurotological signs demonstrated at the bedside, that a peripheral vestibular damage is present, signs of cerebellar involvement were revealed after a few days when the caloric test was performed. In this case, crucial for the stroke diagnosis was the neurological examination.

Three other patients with cerebellar stroke had the unilateral or bilateral gaze-evoked nystagmus. Two of them had unilateral or bilateral FFS. In two patients with cerebellar stroke additional neurological signs were present. In one patient neurological signs were not present, the only neurotological sign of central impairment was gaze evoked nystagmus. On the basis of this sign, the CT imaging was performed and stroke was revealed. Therefore, we underline that gaze evoked nystagmus is an important sign of the damage of central vestibular pathways (in this case probably cerebello-vestibular connections).

The first patient with mesencephalic infarction, had typical symptoms: spontaneous torsional ny to the left, skew deviation with the lower R eye, simetrical caloric test, body inclination to the R. Additional neurological sign were present.

The second patient with mesencephalic infarction, a young woman of 28 years, only a spontaneous torsional nystagmus to the right was present together with the head tilt to the right. None of additional neurological signs were present. In this patient, the suspicion of brain stroke was raised only on the basis of neurotological signs, and with MR imaging, the suspicion was confirmed.

In patients with pontine infarction the central vestibular signs were typical: INO and abducens paresis with gaze evoked nystagmus to the opposite side.

The greatest importance of central vestibular pathways impairment recognition is in patients who don't have additional neurological signs. This was the case in our two patients (one with cerebellar and one with mesencephalic stroke). Grace to this recognition, the proper therapy was immediately administered and all possible risk factor were explored in order of second stroke prevention.

Kattah and coworkers (17) introduced the acronym of HINTS for head impuls, nystagmus and test of skew, as the most important steps of bedside oculomotor examination (in differentiation between the peripheral vestibular impairment and stroke). The group of Kattah and coworkers observed the skew deviation by means of prism cross cover test. In our patients skew deviation judgement was not done by prism cross cover test, therefore we can not comment their finding of skew deviation. However with a small number of patients with brainstem lesion, we would not expect a greater percentage of skew. We confirm the importance of the given acronym. In our patients, besides the gaze evoked nystagmus, an important diagnostic sign was FFS which discovered cerebellar dysfunction.

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Chapter 69

EATING HABITS, FALLS AND STROKE RISK

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INTRODUCTION

Ischemic stroke (IS) the most common type of stroke accounts for 70% to 88% of all stroke events (Ayala et al., 2002: 1197-1201; European Stroke Organization, 2008: 457-507; Brott et al., 2011: e464-e540; Chen et al., 2013: 91-95). As the commonest neurological disease, ranking only behind heart disease and cancer represents the third largest cause of death and leading cause of long-term disability (Allen and Bayraktutan, 2008: 105-116; Planjar-Prvan, 2010: 3-8; Furie et al., 2011: 227-276). Before menopause women more rarely suffer from stroke, but difference among the elderly become blurred (Thom et al., 2006: e8-e151). IS most often occurs in the seventh and eighth decades of life, whereby about ten percent of IS occurs at age of 45 (Nedelchev et al., 2005: 191-195).

According to the American Heart Association, 795.000 people experience a new or recurrent stroke each year in the United States (Go et al., 2013: e6-e245). It is common for the patients who experience an IS to have both modifiable and non-modifiable risk factors for stroke (Khan et al., 2009: 62-7) because evolution of atherosclerosis and IS as clinical events are related to several modifiable and non-modifiable risk factors and that lowering levels of these factors result in benefit. Also, the blood lipid categories include a comprehensive list of lipids known as factor that can accelerate evolution of atherosclerosis (Ding, 2008: 27-31) or are protective and, lower the cardiovascular disease risk (Cuevas et al., 2000: 143-8; Brown and Hu, 2001: 673-86). Atherosclerosis is most frequent reason for the non-cardio embolic ischemic stroke (80%), while 20% from ischemic stroke are cardio embolic, and most frequent reason for their appearance is atrial fibrillation (European Stroke Organization, 2008: 457-507; Brott et al., 2011: e464-e540). In the general population and patients cohorts, risk factor profiles changed with increasing age (Andersen et al., 2010: 2768-2774; Berry et al., 2012: 321-329). A review from 2012 (Gayle et al., 2012: 244-250) reveals that nutrition can

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impact factors that increase the risk of stroke. Similarly to that, in a guideline (Furie et al., 2011: 227-276) and a case-control study (O'Donnell et al., 2010: 112-123) a healthy diet, could substantially reduce the burden of stroke (European Stroke Organization, 2008: 457-507) particularly making simple lifestyle modification (Allen, and Bayraktutan, 2008: 105-116) and/or with effective risk factor management (Gordon et al., 2004: 1230-1240; Furie et al., 2011: 227-276; Gayle et al., 2012: 244-250). Therapeutic lifestyle modifications to reduce high blood lipids and thus decreased stroke risk are based on decreased consumption of saturated fat and cholesterol (Gordon et al., 2004: 1230-1240) including moderate consumption of red meat, sausages and processed products (Lichtenstein et al., 2006: 82-96).

It is recognized that changing dietary habits of ethnic groups in Europe (Penelope and Santosh, 2008: 203-15) from a public health perspective are the basic intervention measures in concept of an integrate approach for prevention of main non communicable diseases such as cardiovascular disease, including stroke (European Public Health Monitoring, 2011). In Finland the diet (particularly fat consumption) has changed and these changes have led to a major reduction in population serum cholesterol, blood pressure levels and ischemic heart disease mortality (Puska et al., 2012: 245-251).

The Former Yugoslav Republic of Macedonia (henceforth Macedonia, or the Republic of Macedonia proclaimed its independence in 1991 when the transition period officially started was one of the poorest republic in former Yugoslavia and now is one of the poorest countries in Europe (Shukarov, 2011). In the current situation the Republic of Macedonia (RM) is a particularly significant example of the redefinition of the state's economic role with disastrous socioeconomic results. As a result, present contrast between the local structures and a nation state-centric viewpoint of society, the effect of diverse socioeconomic policies, administrations, and/or organizations as elements of a system give rise to reflect the dynamic nature of the consequences of less power to regulate socioeconomic processes (Gjorgjev et al., 2006: Shukarov, 2011; Trenchov, 2012: 1-12). In general the transition period in Macedonia was considered to cause changes in lifestyle and health risk behaviors among Macedonian people (Macedonian Health Ministry, 2014: 1-30). An unhealthy diet as consequence of a transition in lifestyles that leads to increased risk of non communicable disease was noticeable (Macedonian Health Ministry, 2004: 9-10). Actually, dietary habits and physical activity levels monitoring as basis for new health promotion strategy to reduce non communicable disease mortality in Macedonia were prominent themes throughout the several investigations (Kosevska, 2004; Simovska-Jarevska et al., 2012: 370-374; Simovska-Jarevska et al., 2013: 20-30). In the background of the health determinants (Macedonian Health Ministry, 2014a), the results obtained from the national "cross-sectional" studies in the Republic of Macedonia with the aim to analyze and evaluate data for monitoring of eating habits in 2011 (Simovska-Jarevska et al., 2012: 370-374) and data on eating habits related to socioeconomic status of the participants in 2012 (Simovska-Jarevska et al., 2013: 20-30), indicate changes in eating habits (Simovska-Jarevska, 2012: 370-374) and socioeconomic difference (Simovska-Jarevska et al., 2013: 20-30) in educational levels and income of the consumption of the main health related foods of population aged 10 to 64 years. Regarding consumption of meat and meat products, most of the study participants consumed chicken and pork, less fish, little lamb and veal; too participants with higher education and higher income, more frequently used fish and beef meat (Simovska-Jarevska, 2013: 20-30). In literature, a differences in eating habits as matter of faith also was observed; Christian believers have experience stroke more frequently than Muslim believers as results of pork meat consumption

(Kosevska, 2004; Ejub et al., 2004: 33) and/or the type of fats in meats (Petrovic-Oggiano et al., 2011: 5-11).

On the basis of these findings of this literature review, the aim of this prospectively case-controlled study was to compare the possible adverse health effects of two different diets due to religious beliefs (consumption or not of pork meat, converted simply “users” versus “non-users”) on stroke risk and to determine whether the changes in blood lipids levels in patients who had an acute phase of first-ever ischemic stroke disease (FIS) and normal control subjects were different between users and non users in both study groups.

MATERIAL AND METHODS

Participants

The study was conducted in Tetovo region in RM, including eligible 425 participants aged from 18 to 87 years of age, with a clinical assessment, a physical examination and with determination of lipid profile from a fasting blood sample measured at entry and dietary history was noted. The protocol was approved by the authority at Clinical Hospital Tetovo. All participants gave written informed consent. The participants who withdrew before completion of the study or who did not have all the required data to derive a response status were classified as no responders and didn't take part in the present study. The clinical study conducted according to good clinical practices. All eligible participants were randomization was performed centrally according to a computer-generated scheme and was stratified by age, gender, religion, eating habits and blood samples collected on admission. In an exploratory analysis, accessibility participants based on the initial examinations (the clinical features and neuroradiological findings) were grouped into two study groups: patients with acute FIS (EG) and normal control subjects (CG), but analysis was done by dietary habits stratification. Disparities in eating habits as matter of faith was defined as the differences in pork meat consumption based on religion dietary restrictions. For objective assessment of the probability of first stroke episode for two types of diet due to religious beliefs assessment of dietary habits was conducted using self-designed questionnaire for the purpose of our previous study (Kamberi and Kamberi, 2012: 181-8) and a physical and neurological examination. Considering the popularity of lifestyle risk factors for IS to simplify the expressed results of our study, according the assessment of the frequency of consumption of various meats: beef/pork, regular consumer of pork meat were the first “users” subgroup included respondents who were once every day, once a week, once a month or once upon a time, whereas regular consumer of beef meat were the second “non-users” subgroup included respondents who never before consume pork meat. Detailed description of the design and methods and of its recruitment procedures and main results has been published (Kamberi et al., 2011: 68-75; Kamberi and Kamberi, 2012: 181-8).

In brief, the present study included a total of 231 patients (126 men and 105 women with an average of 65.7 years) with clinically and imagining verified diagnosis of acute first-ever ischemic stroke disease (FIS) at the Department of Neurology within Clinical Hospital in Tetovo, a secondary care teaching hospital, who were hospitalized between September 1, 2008 and August 31, 2010. The control group consisted of 194 randomly selected healthy subjects with similar gender representation without clinical and anamnesis data for stroke and

related clinical entities. Patients were verified with acute FIS using a detailed taken anamnesis, clinical detailed neurological examination and by means of computed tomography (CT) and/or nuclear magnetic resonance imaging (MRI) of the brain.

Data Analysis

Demographic and baseline characteristics of participants were summarized by descriptive analyses stratified by age, sex, religion and cohort of the frequency distribution of beef and/or pork meat in relation to FIS onset. Also, lipid profile was a priority measure in this study.

The fasting lipid profile was evaluated within days after the stroke onset or at entry and consisted of plasma concentration of total cholesterol (total-chol) (< 5.2 mmol/L, > 5.2 mmol/L,) triglycerides (Trig) (< 1.70 mmol/L, > 1.70 mmol/L, low density cholesterol (LDL-chol) (< 4.1 mmol/L, > 4.1 mmol/L) and high density cholesterol (HDL-chol) < 0.9 mmol/L.

The type of eating habits of participants due to religious beliefs dichotomized as consumption or not of pork meat, typified as simple “users” versus “non-users” and the consumption of pork meat was analyzed by randomization strata into “Yes” and “No”.

The age, gender and religion of participants were the statistical independent variables. The age of participants were coded into five groups less than or equal to 49, between 50 and 59, from 60 and 69, between 70 and 79 and equal or greater than 80. The participant's doctrine were divided into the categories Muslim believer and Christian believer.

Data analysis was carried out using SPSS statistical software, version 17.0. Numerical data was expressed as mean $\pm$ SD. Patients and control subjects variables were compared by t-test for independent samples and unpaired test for univariate analysis. Confidence intervals were computed for all variables. Statistical differences were determined mainly through comparison of confidence intervals to assess consistency of associations and correlations were considered statistically significant if the p value was < 0.05 . All statistical tests were two-tailed.

Study Limitation

In this selected cohort, data were weighted to reflect the selected characteristic of population in Tetovo region. According to the official census figures of 2002, Macedonia's population consist of a majority of ethnic Macedonians (64.18%, predominately Orthodox, with a small Muslim minority), followed by ethnic Albanians (25.17%, predominately Muslims, with a smattering of Catholics and Orthodox), plus other ethnic minorities (Macedonian State Statistical Office, 2005). There most of Macedonian's ethnic Albanians were and are concentrated in the western part of the country, and in certain municipalities (principally around Tetovo and Gostivar, an administrative area of Pollog). There Albanians represent the majority population (Trenchov, 2012: 1-12).

RESULTS

During the two years period, data were collected from eligible 425 residents from Tetovo region as study participants. The gender of the patients was presented in 126 (54, 55%) men and 105 (45, 45%) of patients were female. We found statistically non significant differences (Pearson Chi-Square = 0.523, $p = 0.470$) between patients and control subjects in the

performance of the gender (Table 1). The age at which FIS occurred was no significantly higher in men (66.17 ± 9.48 years) compared to women (65.14 ± 10.097 years). The mean age of the patients was 65.70 ± 9.76 years. Normal control subjects were in the group whose average was the youngest, 52.09 ± 14.49 years. These differences in the average age among participants normal control subjects and patients was confirmed statistically ($t = 11.35$, $p = 0.000$), so we can conclude that the age of patients with FIS has significant impact on onset of disease.

The study participants were dichotomized into “users” and “non users” and observed after dividing they into age subgroups presented in Table 2. The youngest patient was 37, and the oldest was 87 years old. The mean (median) value indicates that 45 (19.48%) of patients were in the age group of 50-59 years, 172 (74.46%) of patients were older than 60 years, while only 14 (6.06%) of patients were in the age group of 18-49 years.

As depicted in the table 2, regarding consumption of meat beef and/or pork at the 425 participant, most of the study participants consume beef and never before pork 312 (73.41%). Study results showed that 113 (26.59%) of participants from all age groups regularly consume pork meat, most of them 39/113 (34.51%) were from 50 to 59 years. With aging, the frequency of users decreased, 19/113 (16.81%) were from 60 to 69 years and 27/113 (23.09%) were from 70 to 79 years, while 7/113 (6.19%) were over 80 years.

Compared with the results obtained in all age groups, a great part of patients with FIS were users in the age group of 50 to 59 years 14/231 (6.06%), from 60 to 69 years were 12/231 (5.19%), and 21/231 (9.09%) were from 70 to 79 years, while 5/231 (2.16%) were over 80 years.

Table 1. Distribution of the study groups by gender

	Control	Patients	
Gender	subjects	with FIS	Total
	(n, %)	(n, %)	(n, %)
Male	99 (51.03)	126 (54.55)	225 (52.94)
Female	95 (48.97)	105 (45.45)	200 (47.06)
Total	194 (100)	231 (100)	425 (100)

Table 2. Dividing the study groups into age subgroups

Age	Control subjects		Patients with FIS	
Groups	Total	Users	Total	Users
(years)	(n, %)	(n, %)	(n, %)	(n, %)
18-49	79 (40.72)	17 (8.76)	14 (6.06)	4 (1.73)
50-59	65 (33.51)	25 (12.89)	45 (19.48)	14 (6.06)
60-69	26 (13.40)	7 (3.61)	83 (35.93)	12 (5.19)
70-79	20 (10.31)	6 (3.09)	72 (31.17)	21 (9.09)
80 >	4 (2.06)	2 (1.03)	17 (7.36)	5 (2.16)

Table 3. Sample characteristics relating to pork consumers

Variables	FIS` s disease		Total
	Yes	No	
Users (n, %)	56 (24.24)	57 (29.38)	113 (26.59)
Non-users (n, %)	175 (75.76)	137(70.62)	312 (73.41)
Total (n, %)	231 (100)	194 (100)	425 (100)

Table 4. Estimates of the relative risk between two study cohorts

Type of study	Value	95% Confidence limits	
Case-Control (Odds Ratio)	0.769	0.500	1.184
Cohort FIS	0.884	0.716	1.09
Cohort CG	0.8677	0.923	1.439

There were 79 normal control subjects whose age was less than 49 among them, 17 (8.76%) were users. The age of 65 normal control subjects was between 50 to 59 years of which 25 (12.89%) were users. There were 26 normal control subjects with age 60-69 of which 7 (3.61%) were users. The age of 20 normal control subjects was between 70 to 79 years of which 6 (3.09%) were users. There were 4 normal control subjects with age greater than or equal to 80 among them 2 (1.03%) were users.

To compare the probability of first stroke episode for two types of diet due to religious beliefs (consumption or not of pork meat, dichotomized into “Yes” and “No”) between patients with FIS and normal control subjects according to food frequency data, all analyzed participants were categorized into users and non-users, while an about equal number of users. The contingency Table 3 displays the frequencies for each exposure.

In both groups dominate frequency of non-users. For these differences in the disease onset were presented in Table 3 we calculated odds ratios for the association of ischemic stroke with selected dietary habits; employing Chi-square test (table 4).

The odds ratio, show in table 4, provide an estimates of the relative risk. This estimate indicates that the odds ratio of FIS disease was 0.77 times higher in the users group; but the odds ratio was low with tightly confidence limits (95% CI = 0.500-1.184).

The participants with Christian believer were 122 and among them, 113 (92.62%) were users, while 9 (7.38%) were non users. The participants with Muslim believer were 303 and among them, not have users. Association between the consumption of pork meat and faith of patients with FIS is shown in Figure 1. The odds ratio from example analysis of crosstabs by chi-square statistics has a value (OR = 0.23, 95% CI = 0.009-0.061) suggesting the relative risk. FIS` disease was with more number of users in Christianity cohort, but according to the low value of OR with tightly confidence limits we can conclude that the association between these two parameters was weak and statistically insignificant ($p > 0.05$).

Additional analysis was performed to compare the lipid profile between patients and control subjects. The fasting lipid profile was evaluated within the level of total cholesterol, triglycerides, low density cholesterol and high density cholesterol in patients compared with control subjects which was dichotomy as users and non-users.

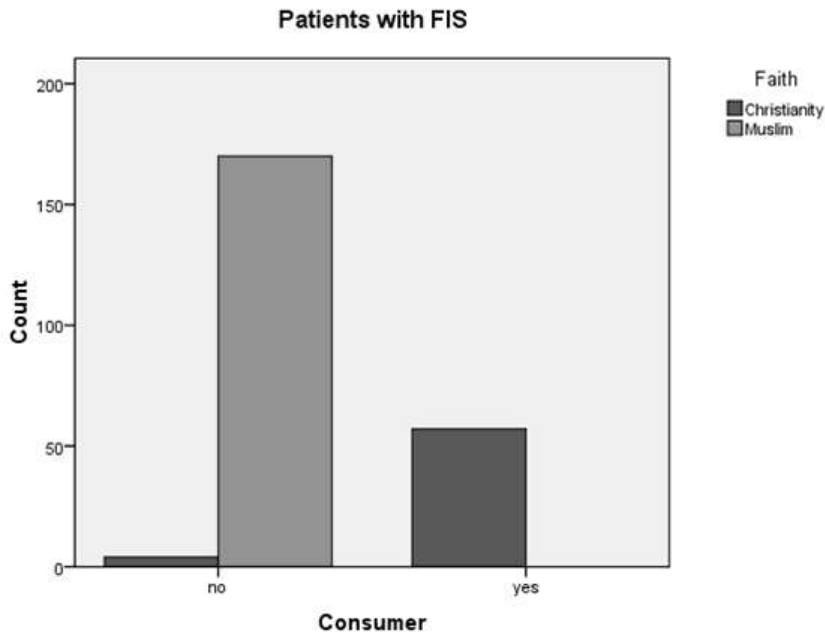


Figure 1. Association between the consumption of pork meat and faith of patients with FIS.

Table 5. Laboratory lipid profile results: comparison between patients with FIS and normal control subjects

	Groups		
Variables	Patients with FIS	Control subjects	Statistical
	(n = 231)	(n = 194)	analysis*
Trig (mmol/L)	1.65±0.66	1.70±0.79	0.526
LDL-chol (mmol/L)	3.87±0.87	3.18±0.92	0.000
HDL-chol (mmol/L)	1.06±0.25	1.19±0.44	0.000

*-test for independent samples. All values are Mean ± SD and data are based on available measurements, including triglycerides (Trig), low density cholesterol (LDL-chol) and high density cholesterol (HDL-chol).

The plasma lipid profile was similar in users and non-users in both study groups except for the level of HDL-chol, which a significant difference in the level of HDL-chol was only observed in controls subjects between users and non-users.

Table 5 shows the distribution of respondents by normal control subjects and patients in terms of obtained results of lipid profile.

When the mean values of plasma lipid profile was compared between the patients and controls, significant changes were observed regarding mean values only for HDL-chol and LDL-chol. In patients with IS, the mean level of HDL-chol tended to be higher with a significantly higher mean level than in control subjects (1.19 ± 0.44 vs. 1.06 ± 0.25 mmol/l; $p = 0.000$), whereas in control subjects, the mean level of LDL-chol tended to be higher with a

significantly higher mean level than in patients with IS (3.87 ± 0.87 vs. 3.18 ± 0.92 mmol/l; $p = 0.000$).

DISCUSSION

Analysis was done by the European Public Health Monitoring 2011 the main non communicable disease such as cardiovascular disease, type 2 diabetes mellitus, cancer, and obesity with their co-morbidities classified in category of nutrition-related disease and in what manner these represent the enormous public health problem (European Public Health Monitoring, 2011).

Recent data on the possible adverse health effect of changes dietary habits (Penelope and Santosh, 2008: 203-15) and attention on a number of factors inherent in modern diets (De Roos et al., 2001: 1233-7), including more fat in the diet (Cuevas et al., 2000: 143-8; Brown and Hu, 2001: 673-86) rather than less has brought increased attention to our work. Prospective studies of red meat consumption (including pork meat) and risk of IS (Misha et al., 2010: 2271-83; Kaluza et al., 2012: 2556-2560) have provided inconsistent results.

Consuming primarily meats with high content of fats, in particularly pork meat, that has been promoted as a model for inadequate eating in Macedonia was associated with cardiovascular disease, including stroke (Kosevska, 2004; Ejub et al., 2004: 33). However, obtained results of our two clinical studies disprove this view. To illustrate the disease effects of eating pork meat we examine the religion dietary restrictions characterized by consuming primarily pork meat in Muslims and Christian's believer from Tetovo region. In the premier study (Kamberi and Kamberi, 2012: 181-8), we measure at baseline contribution of homocysteine levels and meal with high-fat content, particularly pork meat, as variant of traditional dietary fat habits in the acute phase of first-ever ischemic stroke due to the different dietary habits. Conclusion was that meal with high-fat content did not have an impact on homocysteine levels, whereas the limiting pork consumption due religion did not have an impact on stroke frequency. Of all the patients that experienced ischemic stroke, 24.24% were users; suggestive that clear only pork consumption rate was not a risk factor which eventuates in an ischemic stroke event.

The present secondary analysis also examined adherence of their diet's effect on stroke risk by way of exception whether their meal with high-fat content, particularly pork meat, as variant of traditional dietary fat habits as a common and increasing habit may precede high blood lipid levels allusive association with atherosclerosis (De Roos et al., 2001: 1233-7), which eventually link with stroke event independently from traditional risk factors (Furie et al., 2011: 227-276; Gayle et al., 2012: 244-250). The eating habits of these two groups of participants were compared with lipid profile from a fasting blood sample measured on admission. Those who ate pork meat presented a risk of IS occurrence lower than those who never do. This report also concluded that there is insufficient evidence linking eating pork meat with meats with high content of fats and increased risk of IS (Siri-Tarino et al., 2010: 535-546).

Interestingly, Mediterranean populations referred to eat little meat than industrialized nations that eat more meat and have a low cardiovascular disease risk in comparison to their

Northern European counterparts (Vine et al., 2000: 640-4). The primary reason is the so-called Mediterranean diet (Chrysohoou et al., 2004: 152-8; Esposito et al., 2004: 1440-6).

American and European dietary guideline advocated moderate consumption of red meat, sausages and processed meat products (Lichtenstein et al., 2006: 82-96; European Stroke Organization, 2008: 457-507). And yet, from time to time choice is limited by marketable (Dietary guidelines for the Americans, 2010). The dietary guideline established by the Macedonia's Ministry of Health in this year was bearing this in mind (Macedonian Health Ministry, 2014a). This guideline recommended, for example, benefits of the Dietary Approaches to Stop Hypertension (DASH) diets.

In view of the prevailing controversy about the role of eating meat and lipid composition of the meat, no significant association was found between the risk of total stroke and consumption of proteins, including the type of fats (Rosner et al., 2010: 39-45). The results of this study were fully compatible with our obtained results. So, the fundamental difference between Muslims and Christians in Balkan was that the first one has a unified eating habits and the second one a divided different fat meat source that could be solved with their faith because doctrine was involved in their eating traditional sources (Kamberi and Kamberi, 2012: 181-8). Concretely, the characteristics weakness of the type of fats in meat (Petrovic-Oggiano et al., 2011: 5-11) and/or pork meat consumption (Kosevska, 2004; Ejub et al., 2004: 33) were identify as one of the main reasons for important effects of the rise the risk of coronary heart disease, stroke and cardiovascular disease. Traditionally, pork was eaten only by Christians and not by Muslims, but for these authors (Kosevska, 2004; Ejub et al., 2004: 33; Petrovic-Oggiano et al., 2011: 5-11) only Christian believer would be threatened.

In this study, the previously mentioned traditionalism carried out by nature of the pork consumption as traditional eating habit in feeding of subjects for religious reason do not increase risk of first stroke episode in Christians believer. FIS's disease is with high percentage of patients non-users.

The most consistent evidence for this correlation risk estimate by Chi-Square test from contingency table is provided by the comparison of disease rate between Christian's believer who eat pork meat daily or rarely, and Muslim believer, who never eat pork meat.

With regard to the age and results of lipid profile, we found that significant changes between these two groups (EG and CG) were observed regarding mean values of age, high and low density cholesterol. Our results indicate that the disease starts frequently around the sixth decade of life and were similar to finding in more homogeneous populations (Nedeltchev et al., 2005: 191-195; Allen and Bayraktutan, 2008: 105-116).

We analyze possible sex difference in patients suffering FIS because controversy persists regarding gender differences in stroke incidence. Any studies regarding sex difference in patients with IS show the greater prevalence of IS in male (Forster et al., 2009: 2428-2432) but some studies emphasize the importance of stroke in female (Roquer et al., 2003: 1581-1585; Rothwell et al., 2004: 1925-33; Reeves et al., 2008: 915-26). However, other studies do not identify gender difference (Carlo et al., 2003: 1114-9; Sturm et al., 2004: 1327-33). In the presented study we not found a significantly higher incidence of FIS in women. Age of our stroke patients was no significantly higher in men than in women, with an average difference of about one year. According to other authors, women were significantly older than men at their first-ever stroke (Rodica et al., 2009: 1032-1037).

A group authors found that the frequency of consumption of beef and/or pork, increased in the male stroke patients more than in the females (Park et al., 2003: 622-634). In our study,

pork consumption rate also was more frequent in the male stroke patients than in the females of Christian believe. Except these, all fats by nature tend to increase cholesterol, triglycerides, the low-density lipoproteins and the higher-density lipoproteins in the blood. In other words, evidence was accumulating that the high levels of low-density “bad” cholesterol can accelerate evolution of atherosclerosis (Ding, 2008: 27-31). Whereas the higher ratio of the HDL-chol was protective and, lower the cardiovascular disease risk (Cuevas et al., 2000: 143-8; Brown and Hu, 2001: 673-86) so that few studies have compared the lipid profile in patients with IS and controls, but correlation between hyperlipidemia and IS remains controversial. One reason for this was that association was not uniform across the different studies since the lipid fractions probably vary and exerted probably a different influence on stroke risk (Wannamethee et al., 2000: 1882-1888; Iso et al., 2002: 2086-2093). In several studies, IS was associated with total cholesterol (Tirschwell et al., 2004: 1868-1875; Kurth et al., 2007: 556-562), with hypercholesterolemia (Sacco et al., 2001: 2729-2735; Koren-Morag et al., 2002: 993-999), with a low level of HDL-chol (Wannamethee et al., 2000: 1882-1888; Sacco et al., 2001: 2729-2735; Tanne et al., 2001: 2892-2897; Iso et al., 2002: 2086-2093; Koren-Morag et al., 2002: 993-999), or with hypertriglyceridemia (Tanne et al., 2001: 2892-2897; Iso et al., 2002: 2086-2093). However, not all studies reported an association with total cholesterol (Amarenco et al., 2008; Zhang et al., 2012: 1768-1774), or hypercholesterolemia (Iso et al., 2002: 2086-2093), and with hypertriglyceridemia (Wannamethee et al., 2000: 1882-1888; Sacco et al., 2001: 2729-2735). Although one study was show that hypertriglyceridemia was significantly associated with cardioembolic stroke compared to controls subjects (Dahl et al., 2000: 110-117), in another report authors have reported a significant association between hypertriglyceridemia and extracranial arterial atherosclerosis, but not with lacunar infarction or cardioembolic stroke (Iso et al., 2002: 2086-2093). In one study (Cerrato et al., 2002: 653-655) there were no found statistical difference for levels of total-chol, LDL-chol and triglycerides. Similarly, like this study (Cerrato et al., 2002: 653-655) our results do not demonstrate any evidence for an association between the fractions of lipid profile and IS.

Result up until now from the meta-analysis of prospective epidemiologic studies (Siri-Tarino et al., 2010: 535-546), conclude that there was no significant evidence for concluding that dietary saturated fat was associated with an increased risk of coronary heart disease, stroke and cardiovascular disease. Similarly review affirmative participants that following the DASH diet had significantly higher HDL-chol and lower LDL-chol levels compared with the control group, but this diet did not have an impact on triglyceride levels (Gayle et al., 2012: 244-250).

From another point of view, between a numbers of environmental factors that changes dietary habits of ethnic groups in Europe (Penelope and Santosh, 2008: 203-15) and a number of factors inherent in modern diets (De Roos et al., 2001: 1233-7; Hu, 2002: 3-9) consuming primarily meats with high content of fats, in particularly pork meat, and dairy products made of it that has been promoted as a model for inadequate eating in Macedonia (Kosevska, 2004; Ejub et al., 2004: 33) and in Republic of Kosovo (Petrovic-Oggiano et al., 2011: 5-11). A high fat diet is also typical for Finland, Austria and Greece, but in comparison with France, these countries maintains a high rate of coronary heart disease, whereas yet the French have a lower rate of coronary heart disease than many other western countries (Vine, 2000: 640-4). This coincidence has given rise to the fact that unhealthy diet (The report from the United

Nations, 2011) was also not absent in Macedonia (Macedonian Health Ministry, 2004: 9-10; Macedonian Health Ministry, 2014: 1-30).

Conclusive lead of social stratification with higher unemployment rates, deteriorated and unsafe living conditions were and now considered effects of the long time transition process in Macedonia which favored change on the diets of Macedonian people.

The consequence of this modernization modality meal with high-fat content (Kosevska, 2004) and “fast food” in fast food chain (Simovska-Jarevska et al., 2013: 20-30) were cognizable a sign of westernize as well as a common and increasing habit in RM. In the investigations conducted in Macedonia, contribution of the disparity in some of the poorest regions (Kosevska, 2004; Ejub et al., 2004: 33; Simovska-Jarevska et al., 2012: 370-374), the disparities in eating habits as matter of faith (Kosevska, 2004; Ejub et al., 2004: 33; Kamberi and Kamberi, 2012: 181-8), differences in food consumption based on education and/or occupation/income in population (Simovska-Jarevska et al., 2013: 20-30), an unhealthy diet consequence of a transition in lifestyles that leads to increased risk of no communicable disease (Kosevska, 2004; Ejub et al., 2004: 33; Simovska-Jarevska et al., 2013: 20-30) from among authors were interesting and prominent, but still unconvincing. Among other things, there how both socioeconomic changes and westernization impact on the diets of Macedonian people remains unclear.

In this regard, a single report (Shukarov, 2012) explores how the transition changes were still very sensitive question. He presents from citizen's views and perceptions, effects of transition process and economic crisis in Macedonia. There was dissatisfaction, especially among young people and an overall dissatisfaction of the public with the social protection policy in the country. Apart from the problems typically attributable to the transition processes there were the overall deterioration of living conditions. The transition period additionally intensified poverty scale, the Macedonian population in general became more socially vulnerable, as the new poor (unemployed, redundant workers, internally displaced, disabled in the conflict and members of their families). The poverty has a serious impact on the health status of the population, in the case of RM specifically, on the accessibility of health services (Gjorgjev et al., 2006: 1-98). A group authors presents how availability of whole grain bread and eliminating “fast food” are intertwined with most important radical change in the food market and food production (Simovska-Jarevska et al., 2012: 370-374). They also indicate that the promotion of health through improving physical activity and dietary habits should be focused on younger age groups and adults over 50.

Data of this study noticed that the prevalence of consumers was decreased with the increasing age, which means the younger ages are more inclined toward consumption of pork meat. At the same time, in our participants of both study groups, an acceptance of westernize lifestyle was noticeable. These results were not surprising for those familiar with the Macedonian economy and was not experiencing a continuous social unrest.

Thus, in more general terms, the conclusion of our previous (Kamberi and Kamberi, 2012: 181-8) and recent study indicate that the increase risk of IS in individuals from Tetovo region comes indirectly from probability important cumulative effects of role and influence of the daily adaptation in “western” diet, and probability its effect on the major modifiable risk factors for stroke (Gayle et al., 2012: 240-250; Furie et al., 2011: 226-276), that may precede high blood lipid levels and are associated with atherosclerosis (Ding, 2008: 27-31), independently from traditional risk factors (Gayle et al., 2012: 240-250). Moreover, a more detailed description about extension of the westernize Macedonia lifestyle could not be solved

with the faith diet traditional sources and it wouldn't be a bad idea to follow other countries way of doing such.

CONCLUSION

Results of our study show difference of stroke rate hospitalization in genders that difference was paralleled by differences in age of stroke patients.

In conclusion, this controlled study show that as the age increases, frequency of patients with FIS increase at individuals from Tetovo region, while frequency of pork meat consumers decreases. Those individuals not having a different plasma lipid profile although were more expected since to use pork meat in comparison with than those who no consume pork meat. Also the analysis exposes that lipid profile was not associated with IS.

From a religious perspective, Muslims and Christian believers from Macedonia still maintain their individuality. Maybe there was a higher risk from adaptation in "western" diet and/or by the combination of multiple co-existing risk factors, above than high levels of a single factor like consuming primarily meats with high content of fats, in particularly pork meat, that has been promoted as a model for inadequate eating in Macedonia. The process by which stroke risk was induced by adaptation in "western" diet as modifiable behavior, in Macedonia needs further study.

This study recommends promoting the lifestyle and health risk behaviors among Macedonia populations because the demographic, economic and social implications of long transition period were enormous which resulted in poor quality of life and health for an average subject in Macedonia. The impact of these factors has engendered some new phenomenon, not evidence before the 90s, such as tobacco use, physical inactivity, consumption of alcohol and unhealthy diet, four primary behaviors risk factors which were consider to cause a disturbing increase in the lifestyle transition. Also, Macedonia to go by through demographic transition, participation of elderly from 65 years to increase from 7.3% in 1991 to 11.9% in 2012 year. In 2011, 27.7% of population shacks up 75 US dollars monthly, whereas data from 2013 showed a high unemployment rate (31%) from workforce (Macedonian Health Ministry, 2014: 1-30). Literature was in the consensus that respondent's education and occupation/income played a significant role in socioeconomic difference in eating habits (Simovska-Jarevska et al., 2012: 370-374). In populations' lifestyle exist serious problems and these data also provide inside into ways to shape a public health response. As a result, modernity, there's a change in eating habits between tradition and modernity; a new example of the trend nowadays was that a large number of individuals were interested to "fast food". However, a considerable number of them are engaged in health risk behaviors. We need to change the way society view and treats the westernization impact on the diets of Macedonian people.

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Chapter 70

EFFECTS OF PHYSICAL EXERCISE FOLLOWING ISCHEMIC STROKE: IS TIMING AN IMPORTANT FACTOR?

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ABSTRACT

Stroke was reported to be the second leading cause of death and the first leading cause of long-term disability in developed nations. It entails compromised brain function following a disturbance in local blood supply. Survivors of stroke present with persistent neurological defects manifested physically, emotionally, and mentally. Despite immense research, limited therapies exist. Exercise has long been known to provide neuroprotection to ischemic tissue and to improve prognosis of stroke. Early exercise in particular seems to confer neuroplasticity following stroke, mediated by mechanisms such as neurogenesis, angiogenesis, and synaptogenesis. The cause for contention, however, is determining the ideal window of opportunity to maximize the benefits from exercise therapy and minimizing the potential for secondary complications. This article seeks to shed light on some contemporary exercise-mediated therapies and the variables involved. Variables under investigation that could potentially improve prognosis in stroke patients include exercise onset, type, and intensity. Special attention is allocated to eliciting the effects of early exercise at the cellular and molecular levels with the use of human studies as well as animal models.

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INTRODUCTION

Stroke is a major cause of disability and death in developed countries [1] ranking second to ischemic heart disease [2]. Surviving stroke patients present with persistent neurological defects manifested physically, emotionally, and mentally. Approximately 85% of stroke patients experience complications at the hospital, and more than half of them die as a result of complications stemming from immobility [3]. Additionally, 1/3 of patients will die from recurrent stroke within 12 months of the initial stroke, while another 1/3 will be restricted to the most basic activities of daily living (ADL) [4].

Despite immense research, limited neuro-therapies exist.

However, the potential of exercise-mediated therapy for functional recovery post-stroke is well-recognized. Exercise exacts many health benefits and shows promise in providing neuroprotection to ischemic tissue. In fact, exercise therapy is currently widely administered to post-stroke patients. Benefits of exercise in older adults, a population more vulnerable to stroke, include increased chances of survival and healthier ageing in general [5].

Additionally, studies show that training or rehabilitation induces neuroplasticity in regions surrounding the lesion site and the contralateral hemisphere [6]. Despite the prevalence of its use in therapy for stroke patients, an optimal rehabilitation method for stroke patients pertaining to exercise onset, dose intensity, and type remains to be fully characterized [7]. Although clinical research strongly supports early mobilization and training [8], some studies have demonstrated that early exercise may not be beneficial but rather exacerbate brain damage following focal brain ischemia [9-11].

Therein lies the dilemma: how early is early exercise onset in order to be deemed maximally therapeutic to stroke survivors. This review article seeks to discuss the rehabilitative capacity of early exercise in stroke patients, to navigate through the current debate pertaining to defining early in early exercise, and the potential molecular and physiological basis underlying the exercise-mediated rehabilitation post-stroke.

EXERCISE REHABILITATION TODAY

Rehabilitation today recognize that as many as 50-70% of stroke survivors suffer from motor impairments and disabilities such as muscle weakness, reduced mobility, loss of strength and dexterity, and the inability to maintain balance [12, 13]. In reflection, the aims of rehabilitation therapy enable patients to enjoy an adequate quality of life by restoring sufficient function to allow patients to perform the ADL thus maintaining a continued sense of independence [14].

Current Models of Exercise-Mediated Recovery in Clinical Setting

To achieve the most positive outcome, patients are admitted to a stroke unit as early as possible. These units stabilize the patient's medical condition, develop the optimal treatment plan, make efforts to reduce the overall death rate, and to reduce the time spent in the hospital [15]. Once stabilized, rehabilitation becomes the main focus. This process often begins 1-2

days following stroke, taking place either at an in-patient or out-patient facility, or is home-based--all of which occur under the supervision and counsel of a team of physiotherapists, psychologists, occupation therapists, and psychologists. The rehabilitative process comprises of two phases: the early acute phase and the late phase. The early acute phase is essential in reducing secondary complications and impairments while promoting independence. In the late phase, the focus shifts to preventing secondary stroke [16]. However, no process coincides without its challenges. Although contemporary rehabilitative models focus on the acute stroke period primarily to facilitate ADL and functional recovery [17], many patients stop therapy prematurely and are discharged without achieving full recovery. Consequently, almost half of these patients regress in functional mobility within a year [18].

There are many hurdles associated with identifying the optimal rehabilitative strategy for stroke patients. One such factor is the patients' poor capacity for exercise [19, 20]. These patients exert as little as 40% of the capacity of age- and gender-matched individuals with a sedentary lifestyle [21, 22]. Meanwhile, the energy requirements of these patients with hemiparetic gait is increased by 55-100% [23, 24]. Energy requirements may be even greater in patients with neurological disability due to biomechanical inefficiency. Additionally, these individuals have low endurance which can further limit their mobility [17, 23, 25, 26]. In a 50-yard ambulation task, stroke patients experienced dyspnea, progressive slowing, and reduced motor dexterity. Meanwhile, chronic stroke patients exhibited VO₂ levels half that of control individuals, levels which were only sufficient for performing basic ADL. Consequently, performing middle or upper range ADL was exhausting and often impossible. Henceforth, achieving even small gains in fitness levels translates to significant functional gains in stroke patients.

This marks the premise for developing exercise interventions of adequate duration and intensity with the correct onset to stimulate peak aerobic fitness and to facilitate recovery [22].

Clinical Evidence-Based Effects of Early Exercise on Stroke Outcome

For patients with mild to moderate disability following stroke, an early exercise intervention has shown to be one of the most effective forms of rehabilitation [27]. A large meta-review of 21 studies published by Ada et al. concluded that progressive resistance exercise <6 months post-stroke led to significant improvement in patient strength as well as level of activity. Meanwhile, only some improvements ensued in patients in the chronic phase (>6 months). It is thought that the observed difference can be attributed to a greater loss in muscle strength incurred from reduced muscle use and motor unit activity during the chronic phase [28]. This finding poses the important question of whether starting exercise rehabilitation even earlier will lead to an even better stroke outcome.

Overwhelming evidence from clinical studies indicates that exercise initiated within 30 days post-stroke leads to improved functional recovery [19]. In a study of 364 hemorrhagic stroke patients, those admitted to a rehabilitation program within 24 hours of admission performed much better on the Fugl-Myer Assessment Scale (FMA) and Modified Barthel Index (MBI) compared to their control counterparts who received standard hospital ward and internal medical treatment. The most significant gains were observed within the first month [20].

Additional evidence supporting this supposition comes from one of the first large-scale clinical studies looking at the effects of early exercise rehabilitation, AVERT (A Very Early Rehabilitation Trial). This study indicated that patients admitted to a rehabilitative program within 24 hours of stroke onset exhibited positive outcomes. Patients in both the intervention and control group were mobilized out of bed on an average of 18.1 hours and 30.8 hours, respectively, post-stroke with intensity—frequency, length, and level of activity—higher in the intervention group. Their rehabilitative therapy comprised of 10 activities including lying, activities in bed, supported sit, sitting activities, sit-to-stand, standing, early gait, advanced gait, upper-limb training, and other. Both groups received the same baseline standard care. There was no correlation between therapy dose or frequency and the number of adverse events 3 months post-stroke [29].

An additional difference observed between the two groups lay in the capacity of these patients to walk 50m unassisted: the interventional group was able to walk 50m unassisted significantly faster (3.5 days) than the standard care group (7.0 days). Furthermore, Barthel Index and Rivermead Motor Assessment measured at 3 months post-stroke associated the interventional group to better functional outcome [30].

A second study known as the Very Early Rehabilitation of Intensive Telemetry After Stroke (VERITAS) exhibited similar dramatic results when using the same protocol as AVERT (with respect to timing, nature and frequency of intervention) to treat the intervention group. Within 5 days of admission, 74% of the patients in the early mobilization group were able to walk independently, compared to 44% in the standard care group. The former group also exhibited a trend of achieving independence by 3 months with fewer medical complications after adjusting for age and stroke severity [31]. By amalgamating findings from both AVERT and VERITAS, the onset of initial mobilization post-stroke was significantly shorter (21 hrs) in the intervention group compared to its standard care counterpart (31 hrs). The early mobilization onset patients also exhibited a greater chance of acquiring independence by 3 months [31].

An additional study supporting early rehabilitation post-stroke comes from the Post-Stroke Rehabilitation Outcomes Project (PSROP) which looked at 1291 patients in six inpatient rehabilitation facilities. Findings indicated that delaying admission upon onset of stroke, moderate and severe, resulted in lower discharge functional independence (FIM) scores and increased length of stay (LOS). Consequently, the delay time between stroke onset and admission is a significant predictor of discharge total FIM score, discharge motor FIM score, discharge mobility FIM score, and rehabilitation LOS. The greatest gains in improved functional outcome were observed in the subgroup with the most severe stroke [32].

Aerobic exercise has been shown to have significant therapeutic effects in mild and moderately-impaired stroke patients [12]. It can include activities such as rowing, cycling, running, walking, and stepping. For patients with impaired balance, a stationary bike is used.

Patients unable to bear weight on an affected paretic leg are often engaged in aerobic water exercise, which provides some weight support, reducing joint impact loading, while still offering sufficient resistance [12].

A large meta-review of 151 studies published by van Peppen et al. found that the greatest therapeutic outcomes are from a combination of direct, focused tasks of sufficient intensity and early onset [27].

In summary, the discussed studies posit support for a strong association between early enrollment into a physical activity/exercise-based rehabilitation program and improved

functional outcome following stroke. In addition, very early mobilization has shown to reduce medical complications as well as the time necessary to restore functional walking capacity. Similar to early mobilization, late rehabilitation is also somewhat associated with better stroke outcome [28]. It is important to note, however, that clinical studies have many limitations and possible confounding variables. There is very limited data with regards to early exercise effects on patients with more severe stroke, as well as for all stroke patients undergoing exercise immediately following stroke onset (<18 hrs). Similarly, some very early mobilization studies have reached inconclusive results [33]. Furthermore, despite very early exercise generally being promoted [34], it remains somewhat controversial [35, 36]. With the use of animal models such as the well-established rat stroke model, it is much easier to control for variables such as stroke severity or precise location of infarct and to study much earlier exercise onset.

Effects of Late Exercise and Other Factors in a Clinical Setting

In a human clinical study applying learning-based sensorimotor training (LBSMT) beginning 6 months after stroke, improvements ensued with respect to patient independence, fine motor skills, sensory discrimination, and strength. LBSMT is a neuroplasticity-based approach comprised of progression through a set of tasks related to discriminating shapes and textures, vibrations and force, controlling force, holding and eventually moving objects all with the affected hand [15]. After 6-8 weeks, patients that were subject to great intensity of training (i.e., frequency and duration) had much better outcome than their control counterparts who experienced training of lower intensity [37]. Although late exercise is indicated to be effective in maintaining and even improving function and independence through intense LBSMT in stroke patients [37], there is an ever increasing evidence for early and intensive activity to facilitate and accelerate return to unassisted walking and functional recovery in stroke patients [30].

Depending on the onset of late exercise, human studies in general have concluded that brain injury patients can indeed reap the benefits from a late exercise regimen, although such benefits are not as great as if the exercise was started early. Additionally, delaying rehabilitation significantly increases the risks of medical complications [3]. A study supporting late exercise demonstrated that functional benefits in the late stage of recovery post-stroke do ensue learning-based therapy. Additionally, LBSMT for 6-8 weeks was associated with a non-linear positive correlation between training intensity (measured by frequency of weekly visits) and motor functional recovery [37].

TRANSLATING ANIMAL STUDIES TO HUMAN DISEASE

Clinical trials are unequivocally the ultimate translational tool despite being a challenge to design, fund, and conduct. Nonetheless, animal experiments have proven indispensable in the study of human diseases. In the scope of this article, the use of rat models in the study of human stroke treatment research represents a powerful translational variable. Rodent models permit manipulation of various variables while extending control over environmental factors,

all of which have greatly advanced our mechanistic understanding of ischemic stroke pathophysiology.

Despite this progress, gaps exist in translating animal findings to the clinic with respect to applicable therapies.

In the context of exercise-onset dependent recovery from ischemic stroke, timing is proven to be a conundrum that limits our interpretation of the vast animal studies to humans. More specifically, the precise correlation of ischemic time between human and rodents remains unknown. However, it is clear that the tolerable duration of primate and human brain ischemia is considerably longer (6-8 h) than of rats [38]. Secondly, consider that current laboratory studies utilizing rat models are often implementing post-ischemic early exercise between 24-48 hours [39-43]. There is no doubt that the implementation of exercise at 24 hours in ischemic injured rats may not be early enough to simulate human conditions, considering the significantly shorter life span of a rat. This also implicates that the critical period, time course in which the ischemic brain is most sensitive to the beneficial effects of exercise, in animal stroke studies may be different from the critical period in human stroke. Consequently, the age of the animal was found to be a key factor in discerning the neuroplasticity related molecular profile and onset of its expression, which may be an important factor to consider in the translation of these findings to humans.

CURRENT DEBATE ON THE OPTIMAL USE OF EXERCISE

How Early Is Early Exercise

Several factors impact the healing capacity of exercise as related to brain injury. Among them include onset of exercise which can have profound effects on prognosis of surviving stroke patients.

As alluded to previously, many guidelines pertaining to physical therapy and rehabilitation for stroke patients recommend early physical activity and ambulation at the least. As indicated above, clinical studies are applying therapy to patients as early as 18hrs post stroke [29, 30, 44, 45].

Concomitantly, researchers have employed animal models such as the middle cerebral artery occlusion (MCAO) rat models to assist in the delineation of the earliest time frame in which exercise therapy is beneficial rather than detrimental. The earliest documented exercise treatment for MCAO ischemic rat models is 24h [42, 46, 47]. Exercises employed include repetitive and motor skill training [42, 46, 48]. Some studies exposed animals to enriched environments constituting various physical activities [46] as well as force animals to use their impaired limb immediately following surgery [49, 50]. One study defined early training from 0-6 days [51]. Studying the therapeutic impact of various onsets of physical activity/exercise on stroke outcome is key to delineating the window of opportunity in which to reap the optimal benefits of exercise-mediated therapeutic intervention post-stroke. Needless to say, this can have profound effect on contemporary exercise-mediated treatment for patients suffering from various brain injury.

Early Exercise May Be Good or Bad

The rising interest in the potential therapeutic implications of exercise-mediated functional recovery in stroke patients has sparked the pursuit of defining the necessary time frame in which to optimize the benefits of exercise. The plethora of studies that have populated this discipline have led to conclusions short of unanimity. Although several studies have substantiated the beneficial effects of early exercise on recovery from cerebral ischemia [52-57] or hemorrhage [58] in animal models, there are some that contradict those findings by suggesting that early training exacerbates brain damage post stroke [9-11]. This section seeks to navigate through some of these studies.

Animal models are an invaluable tool used to simulate various human brain injuries. The MCAO induced ischemic rat model is one such model onto which the stroke outcome of various early training regimens is immensely investigated. In one study, animals that were administered treadmill training 24h post-surgery for one week exhibited reduced infarct volume and improved neurological function. Early treadmill training may mediate recovery of motor function by re-establishing the normal motor patterns during the sensitive period soon after brain injury [42]. Similar results were observed in the intracerebral hemorrhage (ICH) animal model that underwent early exercise training beginning 24h. These animals exhibited enhanced neurological recovery void of increases in hematoma expansion and edema volume unlike animals that underwent exercise after 1 week [59]. Consistently, forced early exercise on a running wheel led to improvements in functional outcome after focal cortical lesions with no change in lesion volume [60].

Likewise, when placed in an enriched environment 24h post-surgery, MCAO induced ischemic rats showed improved functional outcome without sustaining changes in infarct volume [46, 47].

Additionally, early exercise is shown to promote recovery from ischemic stroke in an intensity-dependent manner [61] with mild to moderate intensity proving beneficial and severe exercise intensity proving to be detrimental [62]. It is thought that a milder training intensity, as in treadmill training for 30 min per day [42], promotes reorganization of relevant cortical representation areas leading to motor functional improvements [63].

The aforementioned outcomes support early exercise as therapeutic to recovery from ischemic brain injury in an intensity dependent manner.

Early exercise following cerebral ischemia may not be entirely ameliorating. Some studies have labeled a period immediately after brain injury (0-6 days) as the early phase.

The early phase was identified as a vulnerable period; henceforth, early physical activity implemented here produced a negative outcome in functional recovery, lesion volume, and lesion-induced up-regulation of plasticity-related proteins [49, 64-66]. Additionally, a number of studies have demonstrated use-dependent exacerbation of brain damage in animals with unilateral lesion to the forelimb representation area of the sensorimotor cortex. In one such study, the unaffected limbs of such rats were immediately immobilized for 14 days following cerebral damage to force the overuse of the affected limb. These animals exhibited the largest behavioral deficits and the longest recovery period compared to when the impaired limb was immobilized. Meanwhile, the latter condition displayed only slightly larger and longer-lasting behavioral deficits [50]. In addition to slowing functional recovery, forced overuse of the impaired forelimb for 7 days resulted in expansion of the lesion and compromised functional recovery [49]. Even without forced use of the impaired limb, simply exposure of MCAO

induced ischemic rats to an enriched environment and administration specific training 24h post-surgery was sufficient to exacerbate cortical tissue loss [64] thus suggesting that perhaps early training may indeed exacerbate brain damage [49, 50, 64].

Nonetheless, these studies stress the onset-dependent role of early exercise in stroke outcome, henceforth, placing importance on deciphering the confines of the window in which physical activity is beneficial rather than detrimental to ischemic tissue and subsequent functional recovery and rehabilitation.

Late Exercise in Rat Models

As in the case of early exercise mediated therapy, effects of late exercise therapy on various brain injury animal models pose similar extent of diverse outcomes. Take for instance the study that administered treadmill training to animals one week after MCAO-induced ischemia; no significant changes in infarct volume or neurological function was noted when compared to spontaneous recovery [42].

Additionally, when animals were forced to overuse their afflicted limb a week after lesion to the representative sensorimotor cortex, impaired recovery ensued although without any change in lesion volume [49].

On the other hand, delayed voluntary exercise following TBI (14-20 days) was characterized by up-regulated BDNF and improved cognitive function [51]. Up-regulation was also evident in downstream effectors of BDNF in both the dorsal hippocampus and cerebral cortex [51, 67-69].

MOLECULAR AND PHYSIOLOGICAL BASIS OF EARLY EXERCISE-MEDIATED RECOVERY

Neuroprotective Capacity of Early Exercise and Its Potential Role in Rehabilitative Functional Recovery

To understand how early exercise can potentially mediate or hinder recovery at the molecular and physiological level, a brief description of the ischemic cascade characteristic of stroke as well as TBI is warranted. Despite the difference in their initial insults, TBI and stroke share similar mechanisms that underline their pathophysiology such as excitotoxicity, oxidative stress, ROS, apoptosis, and inflammation [70]. Henceforth, TBI studies have been invaluable to advancing our understanding of effects of early exercise on stroke outcome and vice versa. The ischemic cascade ignited by stroke onset, a process similar to TBI, is a highly complex mechanism. Depending on the severity of the ischemia, brain cells may respond differently. However, despite this variation, there is a general process that all vulnerable brain cells undergo.

Recall that neurons in the ischemic core undergo apoptosis; however, it is the vulnerable neurons/tissue of the peri-ischemic core area (the penumbra) that undergo the aforementioned reversible debilitating energy consuming metabolic alternations. In agreement, this region is marked by elevated glucose metabolism that can last up to 6 h upon reperfusion [71].

Henceforth, the vulnerable penumbra can be potentially rescued through neuroprotective therapies which is the basis for rehabilitation.

Currently, a clinically effective neuroprotectant is yet to be uncovered, although exercise-mediated recovery is a frequent recourse. The rehabilitative capacity of exercise training on stroke patients is widely recognized and is applied in many physical therapy programs today. As previously eluded to, many such guidelines recommend early physical/exercise therapy. However, as seen in the previous section, the parameters under which early rehabilitative exercise is deemed maximally neuroprotective and functionally beneficial to stroke patients is yet to be characterized. This section seeks to briefly describe the ischemic cascade characteristic of stroke and the potential influence of early exercise on the cascade.

Metabolic disorder. From a metabolic point of view, the immediate repercussions of oxygen deprivation incurred in acute stroke is impaired aerobic mitochondrial oxidative phosphorylation of glucose, the primary energy source for neuronal activity.

Hereafter, surviving brain cells respond by increasing anaerobic glycolysis (hyperglycolysis), a pathway involved in the initial catabolism of glucose [72, 73]. Hyper-glycolysis is reflected in elevated levels of its key enzymes [74] such as phosphofructo-kinase (PFK), lactate dehydrogenase (LDH), and phosphorylated adenosine monophosphate kinase (pAMPK) as well as up-regulation of glucose uptake transporters 1 and 3 (GLUT1 and 3) [75]. Hyper-glycolysis also contributes to metabolic acidosis via lactate accumulation, a by-product of anaerobic glycolysis [76]. Meanwhile, the mitochondria reacts to this hypoxic crisis by increasing the activity of the rate-limiting electron transport chain (ETC) enzyme cytochrome c oxidase (CcO). Consequently, the mitochondrial membrane potential is hyperpolarized to levels that support non-physiological ROS production upon reperfusion [77]. ROS production has shown to cause cellular damage and death in cerebral ischemia and reperfusion [77]. Henceforth, conditions that support ROS production is not a cultivating environment for vulnerable brain cells [11]. Adding to the metabolic disarray, brain cells exhibit uncontrolled energy consuming activity of ion pumps. This facilitates a change in membrane potential supporting liberation of excitatory neurotransmitters such as glutamate [73, 78]. In essence, ischemia brought about by various brain injuries including stroke, is characterized by altered metabolism wherein energy supply is compromised despite an increased demand for it. This creates energy imbalance apt for oxidative stress leading to neural damage and loss-of-function [11].

Metabolic response to early exercise. It is thought that perhaps conditions that exacerbate the hyper-metabolic milieu characterized in the early ischemic period of stroke, such as physical activity and exercise, may have negative effects on the vulnerable penumbra. Consider that PFK-1, a key AMP-activated glycolytic enzyme isoform found in neurons and astrocytes [79], is increased in response to exercise. Likewise, the active form of AMPK was also found to be increased following exercise thus indicating that exercise drives catabolism to meet elevated energy demands [73]. Furthermore, subjecting animals to exercise training [80] or simply exposing them to enriched environments [81] was sufficient to induce angiogenesis, thus exemplifying the body's need to meet increased energy demands. Clinical studies have also established a significant increase in energy demand to sustain the hemiparetic gait of stroke patients [23, 24]. Henceforth, animals forced to use their impaired limb during the early ischemic period, which is marked by hyperactivity in the already vulnerable penumbra, may further tip the energy balance toward a state of deficiency. This sets the stage for transient episodes of hypoxia and reserved recovery [82-84].

The associated metabolic and neurochemical alterations following injury hinders exercise-induced neuroplasticity-associated molecular changes [51]. Eluding to this, cortical stimulation post-TBI elicits a metabolic response that may further increase cortical degeneration [85]. This finding posits that the brain may be undergoing metabolic changes during the first week post-injury, which can strongly affect the outcome of exercise-mediated therapies [86-88].

Henceforth, immature onset of exercise can divert energy stores inappropriately from the much needed production of synaptic plasticity-related molecules to meet metabolic demands of exercise on an already energetically compromised brain. Exercise increases energy demands (primarily in the hippocampus, motor cortex, and striatum) [89], regional cerebral blood flow [90, 91], and extracellular lactate [92].

BDNF signaling pathways. The resulting energy imbalance facilitates the activation of various brain cell death pathways as well as regenerative efforts following stroke. Neurotrophic factors play a key role as neuroprotectants after cerebral insult. Among them include nerve growth factor (NGF) and brain-derived nerve growth factor (BDNF) both of which promote cell growth and enhance neuronal activity [93, 94]. Although a growth factor, midkine has neurotrophic properties implicated in repair of several tissues and found to be expressed in the early stages of cerebral infarction [95].

The BDNF signaling pathway is thought to be a key mediator of angiogenesis [96], neurogenesis [97, 98], and synaptic plasticity [99] all the while serving as a neuroprotective agent [100, 101]. Additionally, this pathway is known for inhibiting the pathological processes of neurotoxicity, apoptosis, and inflammation [102]. In other words, BDNF plays a critical role in post-stroke recovery. Despite this, the effects of stroke on BDNF production has not been completely delineated. Studies indicate an increase in BDNF production at the infarct and peri-infarct sites at least a week following stroke [103, 104]. Aside from neurons, BDNF is also produced by non-neuronal cells such as endothelial cells of microvessels, microglial cells, and astrocytes in the ischemic brain. Additionally, BDNF production by these non-neural cells is positively correlated with infarct size [105].

The signaling pathway involving BDNF is quite diverse. Mature BDNF is cleaved from proBDNF by tissue-type plasminogen activator (tPA). This change allows BDNF to bind to the TrkB receptor enabling the activation of many intracellular signaling pathways including the Ras/extracellular signal regulated protein kinase (ERK), the phosphatidylinositol-3-OH (PI3K)/AKT kinase, and the Ca^{2+} activated kinase (CaMKII) pathways [106]. These pathways converge to manipulate CREB production, phosphorylation, and function [107] which in turn affects transcription of cell survival genes. The AKT pathway indirectly manipulates CREB by deactivating its antagonist transcription factor FOXO3 known to induce transcription of apoptotic proteins [108], henceforth indirectly promoting cell survival [109] (Figure 2).

Another downstream effector of the BDNF-TrkB signaling pathways is synapsin I, a synaptic trafficking protein expressed in axon terminals, involved in the facilitation of neurotransmitter release, axonal growth, and the maintenance of synaptic connection [110, 111]. Its synthesis and phosphorylation is affected by CaMKII and ERK signaling (Figure 2). ICH studies have indicated that the BDNF-TrkB signaling pathway is activated in the perihemorrhagic area. Significant increases were observed on day 7 which subsided close to normal levels by day 14. This suggests that the BDNF-TrkB signaling pathway may be

involved in the brain repair process, although levels present maybe insufficient for complete functional recovery [112].

BDNF-induced neuroplasticity. Exercise is neuroprotective and can induce neuroplasticity in many CNS disorders including stroke [113, 114]. It is linked to slowing cognitive decay [115], neuronal protection against ischemia [53], enhanced neurogenesis [116], and improved learning capabilities [116, 117] making exercise a viable candidate for improving prognosis in ischemic brain injury [118]. The benefits incurred through exercise are strongly linked to increases in neurotrophic factors such as BDNF [56]. Increases are seen throughout the brain, especially in the hippocampus and posterior cortex [119]. It is thought that persistent BDNF expression is crucial for recovery from ischemic/hemorrhagic stroke, the expression of which can be prolonged through exercise. In fact, exercises such as treadmill training have shown enhanced and prolonged activated BDNF-TrkB pathway in the peri-hemorrhagic areas of ICH-induced rats, suggesting the important role of rehabilitation by treadmill exercise [112]. Increases in BDNF are attributed to enhancement of functional recovery in MCAO animals exposed to enriched environment and exercise [120]. Consistently, voluntary wheel running exercise increased downstream effectors of BDNF such as PI3K, PKB/AKT, CREB, and TrkB in the hippocampus [121].

Exercise has also shown to increase the activity of tPA which is responsible for the conversion of proBDNF to mBDNF. This became evident when inhibited tPA activity reduced exercise-induced effects of BDNF. Subsequently inhibition of TrkB receptor and its downstream signaling effectors ERK, Akt, and CaMKII followed. Furthermore, exercise-induced expression of plasticity markers synapsin I and growth-associated protein 43 (GAP-43) were also reversed.

This finding implicates the hippocampal plasticity effects of exercise to BDNF processing and henceforth, TrkB signalling [106].

It is well known that endogenous BDNF and its downstream effectors are up-regulated and functional recovery enhanced post-TBI when running wheel exercise was delayed for 14 days. However, when exercise was administered sooner (0-6 days) post-TBI, endogenous BDNF levels were reduced and associated cognitive impairment were observed. All the while, sham animals exhibited hippocampal BDNF upregulation proportional to the amount of exercise [51]. In addition to reduced BDNF expression, pCREB expression was also reduced and associated impaired learning ensued with early voluntary exercise (0-6 days) [65]. It is possible that the stress of increased metabolic demand imposed by exercise may have adverse effects early after brain injury by further injuring the already compromised tissue.

According to amassing literature, exercise-induced changes in gene expression are perpetuated via modulation of the BDNF system, promoting cell survival and inhibiting apoptosis as discussed previously. Other interactions that converge onto this pathway include the Ca^{2+} activated kinase, CaMKII, which is observed to be up-regulated during acute exercise [122]. Additional up-regulated genes in rats that underwent voluntary running exercise include those involved in synaptic trafficking (syntaxin, synapsin I, and synaptotagmin), neurotransmitter systems, and other signal transduction pathways [122]. Exercise-induced BDNF-TrkB interaction also activates the MAP-K cascade which leads to downstream phosphorylation of CREB [123] and protein synapsin I [124-126]. Among its many roles, CREB induces transcription of target genes, including BDNK [123] related to long-term plasticity [127] and memory [128].

Exercise and Neuroplasticity: Synaptogenesis, Neurogenesis, and Angiogenesis

As eluded to in previous sections, exercise promotes changes in the brain at a neuroanatomical level. Evidence from both human and animal studies converge to suggest that physical exercise promotes neuroplasticity in certain areas of the brain [129]. In the context of ischemic stroke, recovery of motor function involves relearning of motor skills which is a neuroplasticity-mediated process [130]. This recovery process can occur spontaneously post-stroke but can also be enhanced with appropriate rehabilitation [63, 131, 132].

This section will explore the response to post-ischemic stroke with respect to neuroplasticity and the potential role of exercise as a facilitator.

Neurogenesis. Animal studies have established that voluntary aerobic exercise can induce formation of new neurons within the hippocampus of adult mice concomitant to enhanced learning.

This process, occurring primarily in the subventricular zone (SVZ) and subgranular zone (SGZ) of the hippocampus of the adult brain [133, 134], is hampered in the elderly who are a highly susceptible to stroke [135-138].

In addition to its preventative capacity, exercise can also reverse deleterious consequences of aging [134, 139]. Like exercise, environment enrichment, growth factors, and even pathological process such as ischemic stroke can induce neurogenesis. Experimental stroke in animal studies have shown newborn neuron migration into ischemic brain regions. Similarly, stroke patients have expressed markers associated with newborn neurons in the ischemic penumbra preferentially near the vicinity of blood vessels [140].

As discussed previously, trophic factors are key players in adult neurogenesis. In addition to BDNF, notable trophic factors include basic fibroblast growth factor (bFGF-2), epidermal growth factor (EGF), insulin like growth factor I (IGF-I), and vascular endothelial growth factor (VEGF). For instance, intraverebroventricular administration of BDNF increased neurogenesis in the adult olfactory bulb [141] and striatum [142].

In agreement, BDNF knockout mice failed to show enhanced neurogenesis following environmental enrichment [143]. As seen with the BDNF signaling pathway, exercise is also associated with increased genes expression of FGF [68, 144] and NGF in the hippocampus [145].

Angiogenesis. Angiogenesis and neurogenesis are closely associated processes [146-149]. For instance, it has been demonstrated that new cells of the dentate gyrus associate with blood vessels [146] and respond to vascular growth factors such as VEGF [150, 151]. Additionally, increased adult neurogenesis [152] and a reversal of aging-associated decrease in neurogenesis [153] was observed with peripheral infusion of IGF-1 [145].

Like neurogenesis, angiogenesis can also be induced in the CNS by hypoxia and ischemia seen in stroke [154]. It is well established that physical exercise increases angiogenesis throughout the brain [155-158] which is proposed to be mediated by IGF and VEGF. Running exercises enhance IGF gene expression [159, 160], increase serum IGF [161] and VEGF [162].

Consistently, inhibition of VEGF and IGF-1 failed to show enhanced neurogenesis observed with running [145, 162, 163].

Synaptogenesis. The penumbra is the site of active structural and functional remodeling. It is characterized by factors that induce axonal sprouting [132, 164, 165] and support elaboration of dendrites and spines [166, 167]. Positive factors involved in this rewiring process include: glial-derived synaptogenic thrombospondin 1 and 2 [168] and growth-related proteins such as GAP43, mArCKS, CAP23 and growth factors [169-171]. This process is regulated by factors that inhibit outgrowths such as extracellular matrix factors NOGO [172-174], chondroitin sulphate proteoglycan 64, ephrin A5, semaphorin 3A and neuropilin 1, and EPH receptors and ligands [175]. Interestingly, expression of these factors are temporally related such that growth stimulatory factors precede inhibitory factors after stroke [175].

Window of opportunity for neural plasticity in the post-ischemic brain. As eluded to previously, neuroplasticity can occur spontaneously following an injury as seen when the corresponding cortical area of a transected median nerve of adult owls or squirrel monkeys was completely occupied by new and expanded representations of surrounding skin fields [131].

Such cortical reorganization is no exception following stroke. For instance, adult mice in which focal ischemic stroke was induced in the forelimb sensorimotor cortex showed a re-emergence of forelimb-evoked depolarization from surrounding peri-infarct motor/hindlimb area as well as from the distant posteromedial retrosplenial cortex [132].

The existence of a critical period during which the brain is sensitive to exercise rehabilitation is a recurrent theme throughout this article. This concept was eluded to in the previous section pertaining to onset-dependent effects of exercise in animal models. Recall that proteins regarded as positive factors for neuroplasticity such glial-derived synaptogenic thrombospondin 1/2 [168] and proteins that promote synaptogenesis such GAP43 [170], CAP23 and mArCKS were highly expressed post-stroke. Meanwhile, Nogo-A [172], MAG, semaphorin 3A, CSPG [176], and neurocan [177] are thought to be factors that inhibit axonal outgrowth and sprouting. Taken conjunctively, it is hypothesized that an interplay between positive and negative factors that either promote or discourage neuroplasticity, respectively, might be an important determinant of this critical period.

CONCLUSION

Exercise-mediated rehabilitation therapy is unequivocally beneficial in the recovery of stroke survivors. However, the benefits incurred are variable in the scope of early exercise-mediated therapy. Animal studies utilizing various stroke models, particularly the MCAO rodent model, have investigated this phenomenon in functional recovery and have concluded that benefits can range from significant to detrimental. These findings have fueled the proposition of underlying time-dependent factors that may negatively affect the beneficial outcomes of exercise following stroke. Animal studies have focused on deciphering the underlying mechanisms. Molecular perspective under study include the influences of the changing metabolic milieu following stroke, termed as metabolic disorder, and the dynamic signaling pathways involved in neuroplasticity, the cornerstone of functional recovery. Understanding the time-dependent interplay between these processes can perhaps provide a clue to the nature of the onset-dependent benefits of exercise and therefore advance the field of exercise rehabilitative therapy.

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Chapter 71

STROKE AND TRAUMATIC BRAIN INJURY

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STROKE

Anterior Circulation

- Carotid arteries (most atherosclerotic disease occurs at the bifurcation of the common carotid and internal/external carotid artery)

Posterior Circulation

- vertebral artery turns into the basilar artery
- infarction at posterior inferior circulating artery (PICA) results in Locked in Syndrome
- duret hemorrhage: can be seen with trauma, herniation. Consequence of perforating arteries that feed the brainstem

Circle of Willis

- communication between anterior and posterior circulation
- Posterior Communicating Artery: CNIII affected

High Yield Stroke Facts

- Worst risk factor: age

- Other risk factors: hypertension, elevated homocysteine, CRP (inflammation)
- Treatment: intravenous tPA within 3 hours (contraindication: if on blood thinner, seizure at onset, uncontrolled hypertension)
- Reduce risk of stroke: hypertension meds, aspirin, clopidogrel, dipyridole with aspirin, statins, coumadin, stents (more than 70% occlusion will benefit from endarterectomy)
- Major depressive disorder post stroke: 10%, 30-50% will present with some symptoms of depression
 - Treatment for major depression post stroke: Nortriptyline
- Mechanism of cell death in stroke: necrosis

DEFICITS IN STROKE

Motor

- Hemiparesis contralateral to lesion
- Anterior cerebral artery (ACA) stroke: will predominantly affect distal limbs
- Middle cerebral artery stroke (MCA): will predominantly affect face, proximal limbs

Sensory

- (Spinothalamic- pain, temperature, lemniscal- proprioception, vibration) sensory pathways merge in the thalamus
- hemisensory loss contralateral to lesion
- MCA stroke: face, upper limb sensory affected
- ACA stroke: lower limb sensory

Visual

- Homonymous hemianopsia
- Pontine stroke: eyes will deviate toward the hemiparesis
- MCA, PCA (supplies temporal and occipital lobes) stroke: eyes will deviate toward the lesion

Behavioral

- Arousal mediated by reticular activating system
- Left middle cerebral artery (MCA) stroke: loss of language
- Right MCA stroke: loss of visual-spatial orientation, neglect left side
- Brainstem, thalamic strokes: loss of arousal

STROKE SYNDROMES

Anterior Cerebral Artery (ACA) stroke

- Symptoms: contralateral leg is more affected than arm which is more affected than face

Middle Cerebral Artery (MCA) stroke

- Symptoms: contralateral hemiparesis, hemisensory loss, eyes deviate towards lesion, contralateral homonymous hemianopsia

Common or Internal Carotid Artery (ICA) stroke

- Symptoms: contralateral homonymous hemianopsia, unilateral: alexia without agraphia
- Vertebral/basilar stroke
- Symptoms: no cognitive impairment, aphasia or visual deficits

Midbrain Stroke (Weber's)

- Symptoms: contralateral hemiparesis, CN III nerve palsy

Pons Stroke

- Symptoms: contralateral hemiparesis, homonymous hemianopsia, eyes deviate toward the paralysis

Lateral Medullary Stroke (Wallenberg)

- Symptoms: damage to CN V, VII, IX-X nuclei, no hemiparesis

Posterior Inferior Cerebral Artery (PICA) Stroke

- Symptoms: Horner syndrome (miosis, ptosis, anhydrosis), palate paresis, dysarthria, hoarse voice, trouble swallowing, numb face, no hemiparesis

Locked in Syndrome

- Symptoms: anarthria (inability to speak), quadriparesis, preserved cognitive capacity, vision, hearing, ocular movements
- Mechanism of action: infarction to base of Pons (spares CN III, IV, VI, reticular activating system)
- EEG: normal, no problems with thalamic-cerebral connections
- Remember: they have capacity

Vascular Dementia

- Abrupt onset, Step wise deterioration
- Symptoms: dysarthria, gait impairment, pseudobulbar palsy

Transient Ischemic Attack

- Episode lasts less than 24 hours

Anterior TIA: carotid origin

- Symptoms: amaurosis fugax (transient blindness in one eye), hemiparesis

Posterior TIA

- vertebral or basilar origin
- Symptoms: nystagmus, ataxia, diplopia, transient global amnesia (anterograde, several hours, fully alert, cannot learn new facts, disoriented, intellect, general knowledge and personal information intact)

TRAUMATIC BRAIN INJURY**Basics**

- Most susceptible to injury: inferior frontal and temporal lobes
- Coup injury: an injury at the site of impact
- Contre coup injury: an injury opposite from impact site

Epidural Hematoma

- Location: build up of blood between bone and dura

- Most common cause: middle meningeal artery tear from skull fracture
 - Symptoms: loss of consciousness followed by a period of lucidity with rapid deterioration following
- On CT scan: convex in shape

Subdural Hematoma

- Commonly presents in: chronically ill, elderly, alcoholics (alcohol puts wear on vessels, makes them susceptible to shearing), Coumadin use
- Location: between dura and arachnoid matter
- Most common cause: bridging veins are torn
- On CT: crescent in shape

Subarachnoid Hemorrhage

- Most common cause: tonsillar herniation
- Symptoms: “worst headache”, high intracranial pressure, rapid decompensation
- On CSF: xanthochromia (yellow color in CSF because of hemoglobin breakdown)

Transtentorial Herniation

- Mechanism of action: compression of reticular activating system, CN III compression
- Symptoms: coma

	Bulbar palsy	Pseudobulbar palsy
Cause:	polio, ALS, MG	TBI, ALS, MS, CVA
Symptoms:	no gag reflex, respiratory problems	hyperactive gag, no resp probs Emotional lability
Origin	lower motor neuron	upper motor neuron

Normal Pressure Hydrocephalus

- Symptoms: dementia, incontinence, gait apraxia (symptom most quick to improve and most noticed symptom)⁹
- On MRI: expansion of lateral ventricles, no cerebral atrophy
- Treatment: ventriculoperitoneal shunt

Creutzfeldt-Jakob Disease

- Symptoms: myoclonus, personality change (1st symptom), dementia, rapid course, Fatal

- Mechanism of action: prion infection (protein, no RNA or DNA) -> spongiform encephalopathy
- Prions are infectious and can be transmitted through various sources including: EEG electrodes, brain biopsy, cornea transplant, cannibalism (kuru)
- On EEG: periodic or burst suppression pattern
- In CSF: 14-3-3 protein (4)

Subacute Sclerosing Panencephalitis (SSPE)

- Symptoms: onset in childhood, reaction to measles, rapid, fatal
- In CSF: anti-measles antibodies
- On EEG: periodic or burst suppression pattern

Wernicke (reversible)-Korsakoff (irreversible)

- Symptoms: anterograde amnesia (thiamine deficiency – petechial hemorrhage into the limbic system), nystagmus, ataxia (atrophy in cerebellar vermis), confabulation
- Causes: alcoholism, nutritional deficiency of thiamine, bariatric surgery
- Treatment: thiamine replacement (remember to give thiamine first, glucose second)

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Chapter 72

VIRTUAL EXERCISES TO PROMOTE COGNITIVE RECOVERY IN STROKE PATIENTS

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ABSTRACT

The cognitive rehabilitation of stroke often improves executive functioning through repeated and systematic training of memory and attention exercises, for which virtual reality may be a valid approach. Several devices have been used as visual outputs, foremost Head Mounted Displays (HMDs) and desktop screens. HMDs are usually more immersive than screens. However, they present several shortcomings for widespread use. The aim of this study was to assess the prospect of opting for screen displays as an alternative to HMD within virtual reality (VR) based applications to rehabilitate memory and attention impairments in stroke patients. 17 stroke patients with memory and attention deficits were recruited from the hospital *Centro de Medicina da Reabilitação do Alcoitão*, and randomly assigned to two different groups: a) HMD-based VR; and b) desktop screen-based VR. The patients in both groups underwent a 9-sessions virtual reality (VR) training programme with memory and attention exercises. Patients were assessed before and after the VR training sessions with the Wechsler Memory Scale for memory and the Toulouse-Piéron for attention functioning. The results showed increased working memory and sustained attention from initial to final assessment regardless of the VR device used. These results suggest better functional independence following VR-based intervention and support the use of non-expensive displays as an alternative to high-end setups commonly used in VR applications devised for rehabilitation purposes.

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INTRODUCTION

Stroke is a major cause of death in the developed countries and can result in severe cerebral lesions, which are accountable for motor disabilities and cognitive impairments, resulting in personal, professional and social dysfunctions (1). Most of the impairments reveal themselves in the form of attention and memory deficits that often compromise patients' daily life activities. Therefore, an effective approach devised to rehabilitate these functions should focus on exercising daily life activities through systematic and regular training (2).

In this context, there is much ongoing research to assess the role of neuropsychological rehabilitation in cognitive and motor recovery. The most widely known approaches for cognitive rehabilitation are function-based approaches for neuro-rehabilitation since these are focused on a specific cognitive or motor domain (3). Repetitive practice is also an important aspect in motor and cognitive training, as it improves performance in disabled patients (4).

Exercises devised to rehabilitate motor disabilities or cognitive impairments should observe rehabilitation's trinity: repetition, feedback and motivation (5-8). As an attempt to boost these aspects, virtual reality (VR) exercises are now considered a sound option. VR offers the possibility of endless repetition, providing visual, auditory and haptic feedback; and, because VR platforms are usually perceived as a game, patients are engaged, driven and, as a result, motivated by the exercise (6). In fact, according to Cirstea and Levin (7-8), in a virtual environment training exercises can be considered as more engaging and motivating than the traditional approach.

Also, some authors (e.g., 8) have argued that using VR applications in rehabilitation may benefit training purposes, mainly through the 3D spatial correspondence between movements in the real world and movements in the virtual worlds, which may facilitate real-time performance feedback. While repeating the exercises, patients' senses are provided with feedback on their performance, which can improve it further.

VR therefore seems to promote a more intensive and program-supportive approach to the execution of exercise, providing appropriate feedback to the patient. Also, exercises may be displayed with an adaptable degree of difficulty, making possible the use of non-invasive forms of physiological monitoring. VR, in addition, offers therapists the ability to individualize treatment needs, while providing the opportunity for repeated learning trials and the capacity to gradually increase the complexity of tasks while decreasing therapist support and feedback (9). VR is thus a promising response to current trends towards shorter hospitalization and foster homecare periods (10).

Motor aspects of using VR environments have also been studied. A study comparing movements performed by participants with hemiparesis with virtual objects in VR and real objects in real environments found no differences between the movements performed in VR and real environments, suggesting that this VR technique can be an effective training for rehabilitation (11). Other studies (12) compared training a specific function (making a hot drink) in stroke patients in a real-world vs. virtual world setting. Results suggested that virtual applications can be used for rehabilitation of stroke, but the neural mechanisms underlying performance in a virtual world can be different from those in real-life situations. For example, real-world performance was associated only with motor planning, whereas in a virtual world performance was more associated with praxis. Moreover, a study of arm and trunk movements with kinematics in a sample of stroke patients with hemiparesis found results in

line with previous studies and supporting the effectiveness of VR for clinical interventions with these patients (13).

Typically, VR may be experienced using one of following types of settings: (a) desktop personal computer (PC); (b) workbench; (c) CAVE (CAVE Automatic Virtual Environment), (d) HMD (Head Mounted Display) and (e) screens. PC, workbench and CAVE are usually neglected in rehab studies. On the workbench, immersion and presence levels are reduced and the CAVE represents a financial investment difficult to meet by the majority of research groups. Consequently, HMD and screens are the most currently used displays. The HMD, when associated with a tracking system, allows a 360-degree field of view and 3D stereoscopy, which is considered to be responsible for its effectiveness on immersion. On the other hand, most HMDs are heavy, expensive and, when used for a long time, may cause retinal strain. Screens allow the participation of more than one participant at a time and are not as intrusive as HMDs. However, and because the field of view is limited to the projection area and the tracking system is missing, screens are usually considered to be less immersive than HMDs.

Although the use of VR devised to rehabilitate motor and cognitive impairments is being studied intensively, there is no agreement regarding the best way to engage stroke patients in VR exercises. Thus, the main purpose of this study was to compare memory and attention outcomes between stroke patients in a VR-based training program using either HMD or PC-screen displays.

METHODS

17 stroke patients ($M_{age} = 51$ years old; $SD_{age} = 14$ years; 10 males) with memory and attention deficits were recruited from the rehabilitation hospital *Centro de Medicina da Reabilitação do Alcoitão*. All patients had more than 12 years of formal education. Exclusion criteria were:

- More than 6 months after stroke episode (Figure 1);
- Comorbidity of language disorders;
- Dementia;
- Other previous psychiatric disorders that may have an impact on memory and attention, such as drug addiction behaviors or severe depression.

Participants were randomly assigned to the desktop VR experimental group ($n = 9$; 4 males; $M^{age} = 55.56$ yrs; $SD^{age} = 8.91$ yrs; months since injury, $M = 2.89$; $SD = 3.18$) and the HMD experimental group ($n = 8$; 6 males; $M^{age} = 45$ yrs; $SD^{age} = 16$ yrs; months since injury, $M = 3.5$, $SD = 1.07$). There were no significant differences in gender, age, years of education, and time since injury between these two groups (all p 's $> .05$).

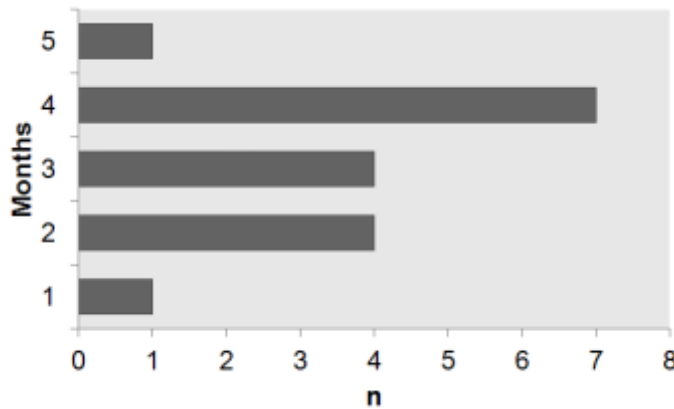


Figure 1. Time since injury.

Measures

Each patient was assessed through a brief screening test, the Mini Mental Examination Test (14) in a version validated for the Portuguese population by Guerreiro and collaborators (15). Memory and attention deficits were considered to exist when Z scores in memory and attention subscales were 2 SDs below the normative value.

During the neuropsychological intervention, our main concern was to stimulate and assess memory and attention abilities, since these are important components of executive functioning. Therefore, each patient was assessed before and after training with the Wechsler Memory Scale – WMS-III (16) and the copy part of the Rey Complex Figure test – RCF (17) for neuropsychological evaluation of memory, and the Toulouse-Piéron test – TP (18) for attention and concentration abilities.

Procedure

This study was carried out at the Psychology Department of the *Centro de Medicina de Reabilitação de Alcoitão*, Lisbon, Portugal. Patients performed a variety of cognitive exercises; either on a HP Intel® Core™2 Quad Processor Q6600 PC equipped with a GeForce GT 220 and a 21'' Asus VE228D screen display (1680 X 1050 pixels of screen resolution) (screen condition), or on the same PC, but plugged to a HiRESeMagin Z800 HMD (HMD condition).

The VR environment for cognitive exercises was developed by our team using Unity 2.5 (Unity Technologies™) and consisted of a small town with a 2-room apartment and a minimarket in the vicinity. The interaction with the VR environment was performed using the left mouse button to move forward and the right to move backwards. The space key on the keyboard was configured as an action button, to grab the objects and interact with virtual objects. The patient's avatar was spawned in the apartment's bedroom, from where they accomplished each session tasks by moving towards the final goal described below. The therapist's role was to explain sessions' procedures and to assess their outcome.

All patients participated in 12 sessions (one session per week). In the first session, memory and attention tests (WMS-III, RCF and TP) were applied. In the second and third sessions, patients acquired computer interaction skills on a training platform. The next nine sittings were used for cognitive training through the VR exercises and final assessment.

Cognitive training consisted of personal orientation tasks, such as the execution of daily living activities of morning hygiene, meal preparation and dressing (i.e., choosing the right clothes to wear), as well as working memory tasks (i.e., buying several items with a certain amount of money), recognition memory tasks (i.e., recognition of outdoor advertisements), visuospatial orientation tasks (i.e., finding a different way to the minimarket) and selective attention tasks (i.e., finding a yellow dressed virtual character) . In the last session memory and attention tests were again applied. An example of these tasks can be found below (Figure2).

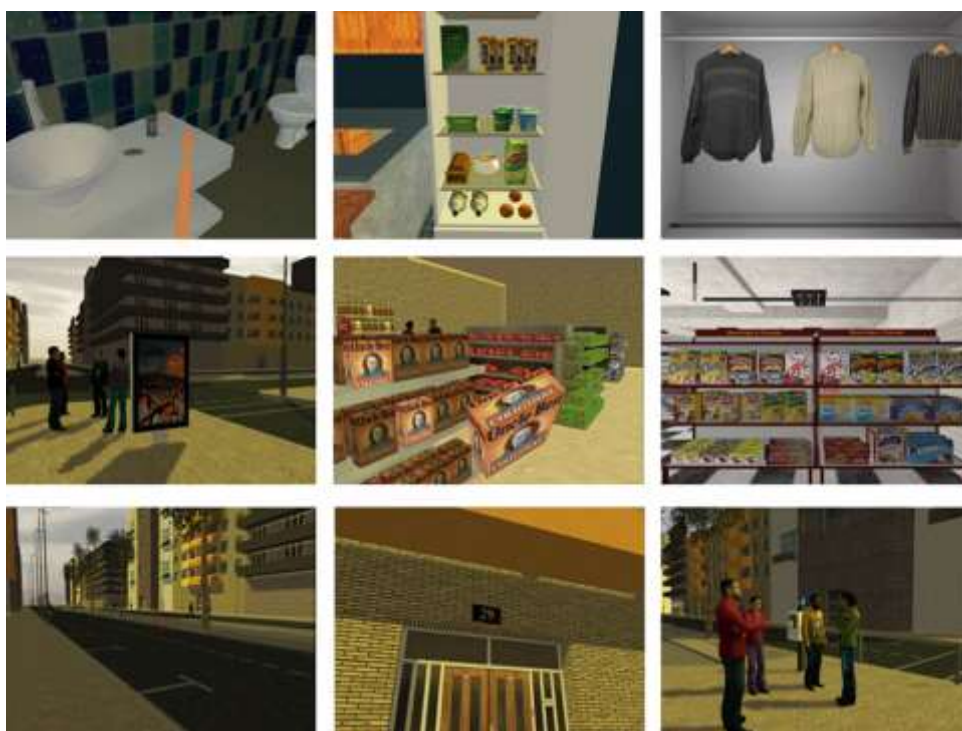


Figure 2. Example of the VR training tasks. The top panel shows personal orientation tasks, the mid panel is for working memory, whereas visuospatial orientation and selective attention tasks are illustrated on the bottom panel.

RESULTS

We tested the effect of type of display with repeated measures ANOVAs with one within-subjects factor (before intervention vs. after intervention) and one between-subjects factor (HMD vs. desktop screen).

As regards memory, we found a main effect of evaluation (pre vs. post-training) on both the WMS ($F(1, 16) = 12.491$; $MSE = 117.813$; $p < 0.01$) and the RCF ($F(1, 16) = 8.676$;

$MSE = 19.709$; $p < 0.05$), showing a significant increase in both WMS scores ($M = 85.71$; $SD = 3.89$ vs. $M = 98.94$; $SD = 3.99$) and RCF scores ($M = 11.41$; $SD = 1.83$ vs. $M = 15.77$; $SD = 2.49$) from initial to final assessment. However, no significant interaction effects were reported between factors (both p 's > 0.05) in either the WMS or the RCF assessments (Figure 3), which indicates that the cognitive improvement did not depend on display type.

Likewise, we found a main effect of evaluation in the TP test ($F(1, 16) = 15.935$; $MSE = 542.598$; $p < 0.01$), indicating that sustained attention increased from initial ($M = 75.69$; $SD = 10.83$) to final assessment ($M = 108.56$; $SD = 16.23$), but no significant interaction effect ($p > 0.05$) between evaluation and display type (Figure 4).

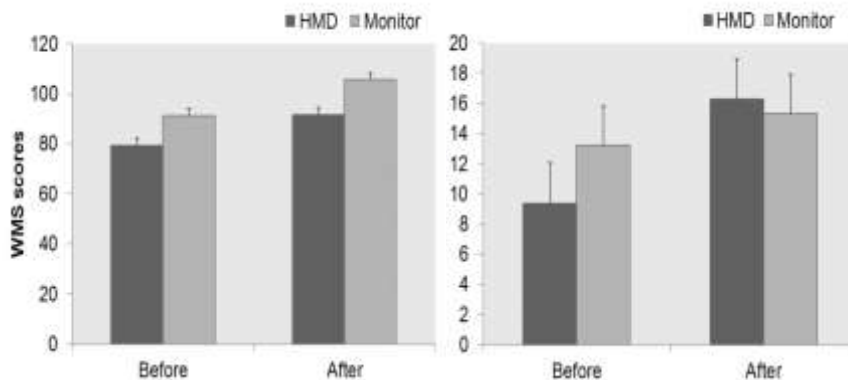


Figure 3. WMS mean scores (left figure) and RCF mean scores (right figure) for each experimental condition.

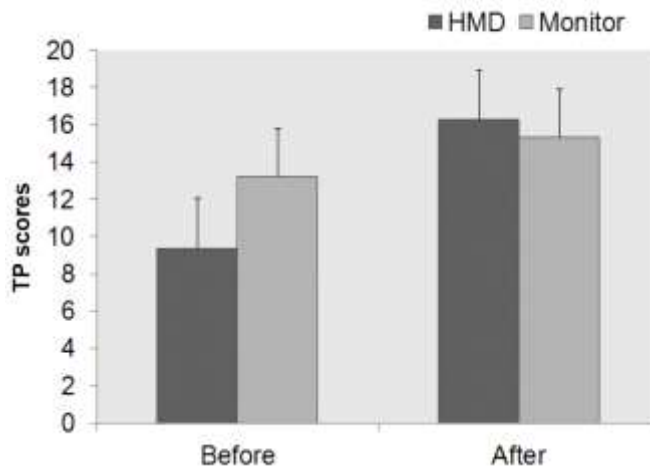


Figure 4. TP mean scores for each experimental condition.

These results suggest that the use of VR environments might be a valid alternative for cognitive training in stroke patients. A control sample is, however, missing. Enhanced working memory and attention and concentration abilities may also imply better functional independence in brain injured patients, which is one of the major goals of neuropsychological rehabilitation.

On the other hand, the results revealed no interaction effects between factors ($p > 0.05$), suggesting that these improvements did not interact with display method of exposure. This outcome may indicate that training cognitive functions in VR settings is, probably, an option to traditional training procedures, and that non-expensive displays, like PC screens, are an alternative to more expensive setups such as the HMD.

DISCUSSION

VR applications have long been applied for rehabilitation purposes. Their use to train cognitive functions is now common. VR applications have several advantages when compared to their traditional counterparts. They are immersive, enable a certain amount of free will and they are ecological sound. One entity that has been working as a propeller for their use is the videogame industry. There are now off-the-shelf graphic engines available that are relatively easy to use and that produce realistic and interactive synthetic worlds. Also, on the hardware side, advances in computer components like CPUs and graphic boards are giving a hand, as they are the associated with output devices such as screens, HMD (head mounted devices), or CAVEs (Cave Automatic Virtual Environments).

Screens and HMDs are usually the therapists' choice (CAVE are expensive and require extra manpower). HMDs are immersive and, more often than not, are coupled with a head tracker that emulates one's head movement in the virtual world. On account of all this, they should be "today's special". However, they are not. They are more expensive than screens, they can cause visual discomfort and, above all, as a stand-alone extra they are difficult to justify for lay users such as patients and therapists. Furthermore, the evolution of screen-based displays has placed screens competing shoulder to shoulder with HMD technology. The quality of both general-purpose TVs and computer displays, allied to first-rate and rather cheap sound systems, enable a decent immersive experience that could only be obtained a few years ago by HMD.

This apparent parity is, however, in need of assessment. Accordingly, this study aimed at comparing two samples of stroke patients that had trained memory and attention functions with either an HMD or a PC-screen display. The results suggested that the observed improvement in those functions was independent from the display type used, which supports the idea that screen-based displays may be a sound alternative to HMDs. However, larger sample sizes and/or a greater number of studies are needed to boost power and be more conclusive.

This is important because in western countries, where the elderly population is increasing and the average age of stroke is decreasing, combined with reduced hospitalization periods, the need for rehab applications that can be handled outside health institutions facilities is paramount. Without the direct support of well-trained caregivers, exercises should be devised to be as straightforward as possible. As too should the equipment employed for the VR experience. Under the same circumstances, i.e., producing equivalent therapeutic outcomes, the choice should go towards cheaper and easier-to-handle devices such as screens.

In addition, this study also generally supports the use of VR exercises aimed at training memory and attention functions in stroke patients. The cognitive rehabilitation exercises used in our program were developed according to a cognitive retraining rationale with a focus on

memory and attention functions (3), and support the focus of that rationale on the repetitive training of specific cognitive skills that can help to recover disrupted functions, leading to better adjustment in personal and social domains.

Indeed, irrespective of the specific aim of the each intervention program, meaningful improvement in patient's everyday living activities must be the major goal of rehabilitation. One suggestion for further studies is that evaluation should focus also on the level of activity and engagement in activities of everyday living (ADLs). For example, level of performance in ADLs could be assessed through the caregivers' opinions that could allow the understanding of personal, professional and social adjustment of patients in basic and instrumental ADLs. Also, controlled trials are needed to compare VR-based interventions with other conventional approaches or even with a waiting-list group.

To conclude, despite the need for further empirical support, VR-based applications offer promising prospects in cognitive rehabilitation. Moreover, evidence from motor aspects of using VR environments, which have been well studied (11-13), suggests that the 3D spatial correspondence between movements in the real world and movements in the virtual worlds can benefit training purposes with VR applications. Another important issue with rehabilitation is the patient's motivation to perform the predetermined exercises. Training in a VR setup is typically perceived more as a game and less as a task, and is therefore more engaging and more stimulating than the conventional methods of rehabilitation.

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Chapter 73

AUTOMATIC NON-CONTACT CATEGORIZATION OF UPPER BODY MOTION IMPAIRMENTS AND COMMON POST-STROKE MOTION SYNERGIES

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ABSTRACT

Upper body motion impairment is a common after-effect of a stroke. We are developing a novel multisensory therapy system that makes use of augmented feedback and is expected to enhance rehabilitation outcomes. The first phase of the project involved developing algorithms to automatically differentiate between normal and impaired upper body motions. Seven healthy subjects performed two types of scripted actions: (a) elbow flexion and extension and (b) reaching over via elbow flexion and shoulder flexion and adduction. Each action was repeated 10 times as each participant felt most comfortable and also 10 times simulating a common post-stroke impaired motion according to the literature and in consultation with a physiotherapist. Principal component analysis was applied to the upper body trajectories during each action, as observed with a Kinect sensor, to extract the dominant modes of motion. Three classification algorithms and up to three motion modes were examined in order to distinguish between normal and impaired motions. A statistical analysis of the Kinect skeletal tracking data vs. manual annotation confirmed a significant bias in the tracking of an elevated shoulder joint. Despite this bias, leave-one-subject-out cross validation experiments confirmed the effectiveness of the proposed methods in detecting impaired motions (accuracy >95%), even when the impairment involved elevating a shoulder. A

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single most dominant motion mode provided sufficient information for the binary classification task. The high accuracy rates validate the use of vision-based pose tracking technologies in identifying motion deficiencies.

INTRODUCTION

Hemiparesis, or weakness on one side of the body, is a common after-effect of a stroke. A well-known body illusion, known as the mirror box illusion, is used in rehabilitation training exercises and has been shown to be effective in restoring mobility (1). During the treatment, the patient enters both arms into a box with a mirror in the middle so that the view of the affected arm is replaced by the mirror reflection of the healthy arm. Simultaneous motion, or attempted motion, of both arms results in artificial visual feedback indicating that it is, in fact, the affected arm that is moving, thus promoting neurorehabilitation.

A closely related body illusion, the rubber hand illusion (2), offers promising potential in strengthening the visual feedback from the mirror box. In this illusion, a plastic arm is placed within view on a table next to the real arm, which is hidden by a screen, and both the rubber hand and the real hand are simultaneously touched by a brush for a few minutes using a rhythmic motion. The temporal synchrony of the seen movements on the rubber hand and the felt movements on the real hand causes the observer to embody the rubber hand so the perceived physical location of the real hand can be shifted towards the seen location of the rubber hand.

Objectives

The main objective of this work is to reproduce and combine the aforementioned illusions in a virtual environment. It is expected that augmenting mirror box therapy with the rubber hand illusion will strengthen the sense of ownership over the artificial visual feedback and will thus enhance the rehabilitative potential. The virtual environment will also enable a wider range of motion than a physical mirror box in which motion is quite constrained.

The project takes advantage of state-of-the-art computer vision and markerless skeleton tracking systems to tackle the engineering challenges and to counter some of the weaknesses identified among many of the virtual rehabilitation technologies (3), e.g., in the user interface or in the interaction methods with the virtual world. The final system is being targeted at an affordable price ($\leq \$200$) to enable wide deployment and home usage. The system is also aimed at being easily configurable so it can be adapted to target a range of mobility impairments.

The skeleton tracking information is processed to identify impaired modes of motions by pre-processing short segments of scripted actions. This information will then be used to modify the profile of subsequent motions in real-time in order to exaggerate impaired motions, for instance by amplifying a reduced range of motion, or exaggerating impaired synergies. Finally, the modified motion is displayed in a virtual environment in real-time and in synchronization with tactile feedback.

Contributions

This paper presents results from the first phase of the project, which focuses on the development of posture categorization pre-processing algorithms and preliminary validation experiments on healthy subjects. Since measuring range of motion deficits is relatively easy via skeleton tracking, the experiments presented here focus on the more challenging task of identifying motion synergies. Impaired motion synergies are a common after-effect of a stroke and refer to the undesirable couplings of human degrees of freedom.

We have also performed preliminary experiments to validate the skeleton tracking algorithm in a specific scenario related to this research. The results identify an inherent bias in tracking the shoulder joint. We provide a short discussion on the consequences of such limitations and how they might affect the assessment of posture and motion.

METHODS

The system employs a consumer depth sensor (a Microsoft Kinect) to capture live video and depth images and to track major skeletal joints of the body in real-time. The PrimeSense™ open source OpenNI and NITE libraries were used to interface with the device. In the final system, touch feedback will be provided via a wearable glove equipped with small vibrating motors, and synchronized with the visual display through a digital I/O board and a digital solid state relay board. The touch feedback interface was not utilised during the experiments reported here as it did not relate to identifying impaired motions.

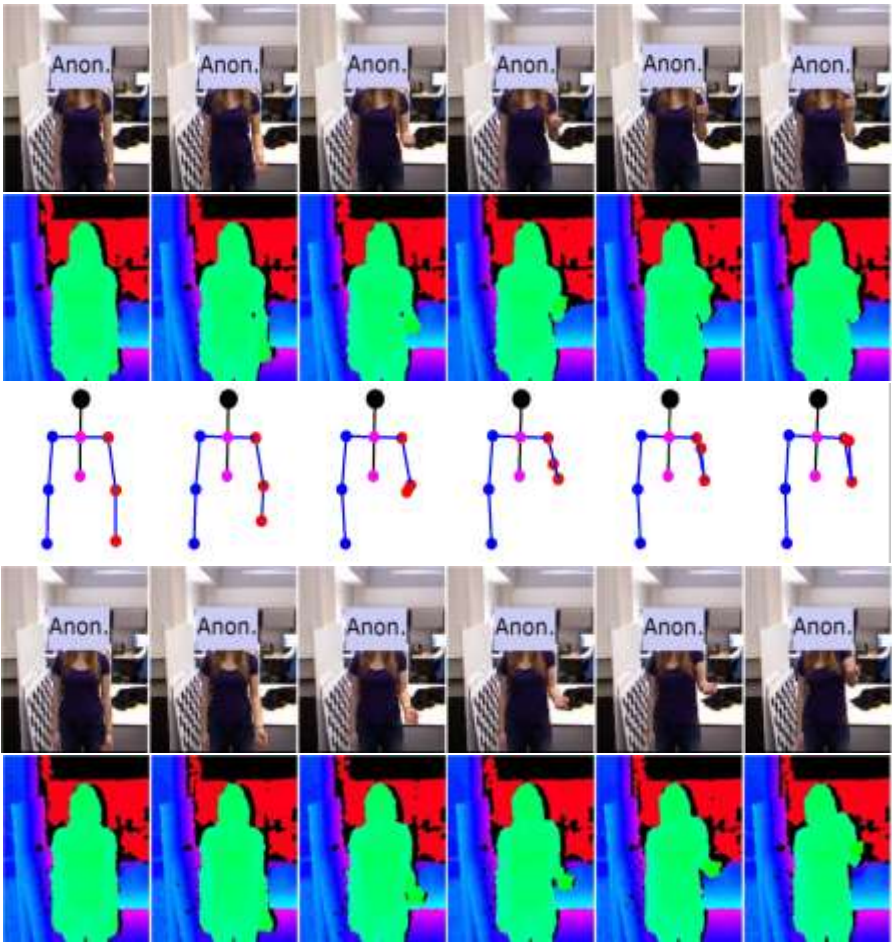
Experimental Dataset

Two of the most common upper limb flexion synergies present in post-stroke patients were selected according to the Chedoke-McMaster Stroke Assessment 2005 and were simulated by healthy adults. The selected actions were (a) reaching over to the opposite shoulder via elbow flexion and shoulder flexion and adduction (see Figure 1) and (b) elbow flexion (see Figure 2). The stereotypical post-stroke movement synergy associated with flexing the elbow is hiking the shoulder, while reaching across the body is typically accompanied by rotating the trunk (4).

Seven healthy adults (4 males, 3 females) were recruited and asked to use their right arm to perform each of the two actions 20 times in front of the sensor, 10 times normally as they felt most comfortable and 10 times simulating the impaired motion. The 3D coordinates of the upper body skeletal positions were normalized into a subject-centred canonical coordinate frame independent of the body size and the viewing angle (5). The 3D displacement of all upper body skeletal joints in consecutive frames was computed.



Figure 1. Sample color and, depth images in six representative frames over two sequences of reaching across. The top row illustrate a “normal” movement pattern while the bottom row illustrate a simulated “impaired” motion synergy of rotating the trunk when reaching across, common among the post-stroke population.



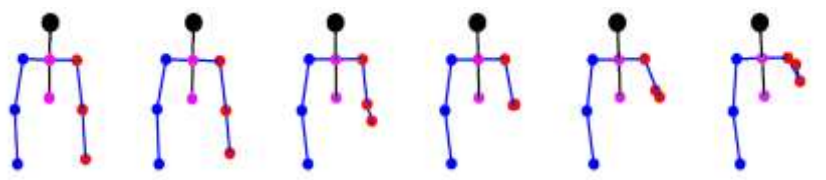


Figure 2. Sample color, depth, and skeleton tracking images in six representative frames over two sequences of elbow flexion. The top three rows illustrate a “normal” movement pattern while the bottom three rows illustrate a simulated “impaired” motion synergy of hinging the shoulder when flexing the elbow, common among the post-stroke population.

Considerations of Tracking Accuracy

As the real-time skeleton tracking system used in the experiments, i.e., the Kinect (6), is a relatively new technology (introduced in late 2010), its accuracy levels and limitations are not fully known and the existing literature on the topic is sparse. Most notably, Dutta (7) evaluated the depth sensing accuracy of the device and, more recently, Clark et al. (8) validated its skeleton tracking capabilities. When compared to a marker-based Vicon motion capture system as the ground truth, these studies concluded that the Kinect provided sub-centimetre accuracy in depth sensing in a sizable workspace (covering a range from 1 to 3 m from the sensor and a field of view of 54°) and its 3D skeleton tracking had comparable inter-trial reliability. However, Clark et al. also observed tracking biases in estimating the position of the torso and the pelvis.

During our experimentations, it was observed that the skeleton tracking library sometimes estimated joint positions that visually appeared as being systematically biased in certain ways. These biases were mostly observed in the tracking of the elevated shoulder joint and were different from the earlier published results reporting biases in the pelvis and the torso. Therefore, a series of measurements were made to confirm and quantify this visual observation. With the growing interest in applying the Kinect and similar commercially available systems (*e.g.*, ASUS WAMI Xtion) in gait and upper extremity mobility function assessment and other applications in rehabilitation, monitoring, and assistive technologies, it is hoped that the results from these experiments will contribute to obtaining a better understanding of the capabilities and limitations of these technologies.

Automatic Categorization of Motion Impairments

The system is expected to observe a human subject performing a short scripted motion, such as flexing their elbow, and to automatically analyze the motion characteristics of the upper body and detect possible impaired motions.

Principal component analysis (PCA) of the displacement vectors was used to compute the dominant modes of motion over the course of each action and the top N PCA dimensions, along with their corresponding singular values, were used as the feature set in a binary classification to distinguish between normal and simulated impaired motions. Seven-fold leave-one-subject-out cross validation was used to train and validate three binary classification algorithms, each separately trained for identifying impaired motions while

flexing the elbow or while reaching across. The classification algorithms included logistic regression (LR) (9), binary support vector machines (SVM) with radial basis function kernels (10), and Random Forests (RF) (11). The SVM and the RF are state-of-the-art machine learning algorithms (12) while the LR is a simple linear classifier used here as a baseline.

Assessing the Skeleton Tracking in Impaired Motions

With an elevated shoulder, it was visually observed that the 3D skeleton tracking repeatedly underestimated the degree by which the shoulder was elevated by a large margin (see Figure 2). To confirm this observation, in seven videos simulating a motion with a hiked shoulder (one of each subject), ten frames were randomly selected for manual annotation. Two human annotators were separately asked to each mark the location of the left and the right shoulder of the subject at each frame via a mouse click and the elevation angle of the connecting lines, i.e., the angle with the horizontal axis in the 2D image plane, was computed.

For comparison, the same angle with the horizontal axis was also computed based on the 3D tracking data. To calculate the angle, the 3D line segment connecting the estimated location of the left and right shoulder joints, tracked in the depth sensor coordinate frame, was first transformed into the color camera coordinates via the default color-depth registration of the Kinect, i.e., a rigid 3D transformation. The 3D line was then projected onto the image plane based on the default extrinsic and intrinsic calibration parameters of color camera.

Ideally (i.e., in the absence of any inaccuracies or biases in the tracking of the shoulder joints) angles computed from the skeleton tracking data would be very close to those from the manual annotations. If the error in 3D tracking is larger than that of the 2D annotations, it is still expected that the angles be without a bias. That is, while the variance of the angles computed from the 3D tracking could be larger, their mean will ideally be the same as that of the manual annotations.

Admittedly, identifying the shoulder joints visually and via user mouse clicks is not always very accurate. Nevertheless, it is reasonable to assume that the human annotators will mark the left and the right shoulder similarly and their annotations will lead to an unbiased estimate of the elevation angle over the 70 frames. Furthermore, the agreement between the two manual annotators was used as a baseline to confirm the validity of the annotations. Various statistical comparisons between the angles from human annotations and the 3D tracking were used to assess the bias in the 3D tracking of elevated shoulder joints.

FINDINGS

The SVM correctly identified simulated impaired motions with high accuracy (~ 96% and 97%), see Table 1. The RF also provided good, albeit slightly less consistent results (98% and 90%). A single motion mode (i.e., N=1, the most dominant motion over the course of an action) provided sufficient information to discriminate between normal and impaired motions.

Table 1. Classification accuracy in distinguishing between normal vs. simulated impaired motion. The algorithm with the best performance in each action category is boldfaced

Action	N	Classifier		
		Logistic Regression	Support Vector Machine	Random Forest
Reaching	1	54.3	97.1	97.9
Across	3	61.4	85.7	97.1
Elbow	1	64.3	95.7	90.0
Flexion	3	59.3	85.0	92.9

The high rates of accuracy validate the use of commercially available and affordable vision-based pose tracking technologies in identifying motion deficiencies. This encourages further work into the development of the fully integrated tactile and visual virtual reality system for inducing sensory conflicts and targeted rehabilitation therapy. Future work includes validation experiments with real (i.e., not simulated) impaired motions through testing with actual stroke survivors, real-time exaggeration or attenuation of undesirable synergies, and human subject rehabilitation efficacy trials.

Verifying a Tracking Bias

The correlation coefficient (ρ) between the sequence of 70 numbers further illustrates the agreement between the two human annotators ($\rho_{h1-h2} = 0.98$) and the disagreement between the human annotators and the 3D data ($\rho_{3D-h1} = 0.20$ and $\rho_{3D-h2} = 0.15$). The smaller coefficients indicate that the angles computed from the 3D skeletal joints not only underestimated the shoulder elevation angle, but also were less correlated with the true elevation angle.

Finally, the Student's t-test and its non-parametric equivalent, the Wilcoxon signed rank (WSR) test, also rejected the hypothesis that the 70 angles computed from the 3D joints were taken from distributions with the same mean (or median in the case of the non-parametric test) as that of the 2D angles, while strongly confirming that the sequence of the two human annotators indeed were from the same distribution.

The NITE skeleton tracking library provides a 3-value {0, 0.5, and 1} confidence level for each estimated joint position, where 1 is the highest confidence and 0 is the lowest. The confidence values of the estimated position of both shoulder joints, provided by the skeleton tracking library, were at the maximum level (i.e., 1) in all 70 frames. In other words, any discrepancy between the 3D tracking data and the 2D annotations could not be attributed to low confidence tracking.

The results in assessing the tracking of an elevated shoulder highlight the need for a better understanding of the algorithms applied in the skeleton tracking libraries and their underlying assumptions if their usage is to become more widespread in gait and posture assessment. It is interesting, however, to note that despite the inability of the system to track an elevated shoulder joint properly, the developed posture categorization system worked well with a high accuracy of 95.7%. This was only slightly lower (1.4 percentage points when using SVM) than the accuracy rates obtained in identifying trunk rotation. Perhaps even the

slight elevation detected in the placement of the shoulder, combined with other cues from the orientation of the shoulder-elbow and other segments, was sufficient in detecting this compensation mode. It is, however, reasonable to assume that better skeleton tracking would improve the overall identification of compensations.

DISCUSSION

When observing short scripted motions, the most dominant mode of motion, as identified by PCA, provided sufficient information to discriminate between normal and simulated impaired motions with high accuracy. Inherent biases were observed in the tracking of an elevated shoulder via the Kinect sensor. The automatic detection of an impaired motion involving an elevated shoulder was only marginally affected by this bias. However, further research in identifying and quantifying such biases is required so that they can be taken into account when designing rehabilitation and health monitoring systems that use the Kinect.

Current work in completing the first phase of the research focuses on experiments with post-stroke survivors with actual motion impairments to validate the results obtained in classifying the simulated impaired motions evaluated here. Future work towards the full implementation of the sensory-conflict rehabilitation system involves the real-time augmentation of impaired motions, duplicating the rubber hand illusion in a virtual environment via real-time tracking and simultaneous touch feedback, combining the rubber hand illusion with the mirror box illusion via real-time motion augmentation, and clinical trials.

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Chapter 74

PATIENT ENGAGEMENT AND CLINICAL FEASIBILITY OF AUGMENTED REFLECTION TECHNOLOGY FOR STROKE REHABILITATION

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ABSTRACT

This chapter presents a study that evaluates patient engagement and clinical feasibility of an Augmented Reflection Technology system for use in physical rehabilitation of the upper limb following stroke. In particular, TheraMem, an extension of the Augmented Reflection Technology, was assessed on its potential to engage patients in meaningful therapeutic exercise. Six patients participated in a total of 24 sessions of upper limb training with the system. Tailored support for patients performing the exercises was provided based on the severity and level of their impairment. Various configurations of the system were evaluated and adjusted to best match the patient's preferences as well as the therapeutic requirements. It was found that all patients were able to successfully participate and complete the TheraMem intervention at a high level of engagement and motivation over the course of the therapy sessions.

INTRODUCTION

The use of virtual reality and other forms of computer mediated visual feedback can be beneficial for patients in the therapy of motor impairments after stroke, for the improvements of arm function as well as in activities of daily living (1).

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Our research group has used computer mediated visual feedback, in the form of Augmented Reflection Technology (ART), to fool users about the properties and capabilities of their hands. Users place their hands in two black boxes where webcams video-capture the hands and transmit the video-streams to a PC. These video-streams are then manipulated and displayed on a screen on top of the boxes (2). Figure 1 illustrates our therapeutic setting of the ART system.

Augmented Reflection Technology allows various ways to visually manipulate what the users see. The fooling of healthy participants into believing that their right hand, displayed on the left side of the screen, is their left hand was extensively evaluated (2,3). In addition the augmentation with virtual backgrounds or 3D models using the ART system is also possible and was previously evaluated (4). Yet another manipulation possibility is the spatial manipulation of the displayed hand by the therapist or operator, which allows change in the position of the user's hand vertically or horizontally in addition to the user's own movement (4).

The TheraMem extension of the ART system, can be described as an augmented reality memory-game incorporating some of the visual manipulation aspects previously described (5). Users move their hand on the floor of the black boxes but experience the video-output on the screen as a three-dimensional environment. In this virtual environment, 12 tiles on each side of the screen are shown.

The task of the users is to try to find matching pairs of plants for the left and the right side by turning exactly one tile for each side. Virtual tiles are turned and the plants are revealed when the user points with a finger or the hand at a tile for a certain amount of time (see Figure 2). The movement of the hand, and the direction that the hand is pointing to, is tracked using a finger-tracking extension specifically added to ART. In addition, TheraMem contains a feature that allows the therapist or operator to amplify the movement of the hand by displaying larger movements on the screen than the user actually performed. The details of this manipulation, the technological background of TheraMem, as well as a detailed study with healthy participants have been previously described (5).

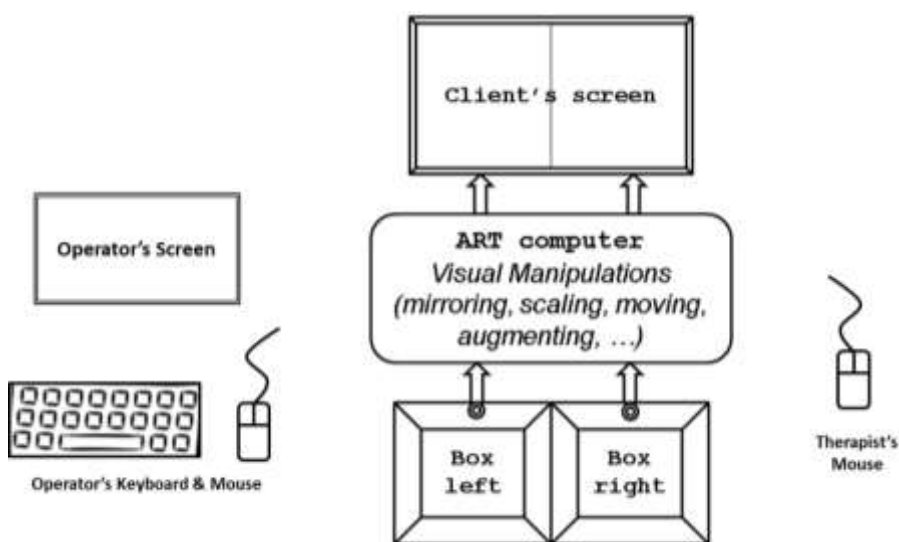


Figure 1. A sketch of the ART system used.

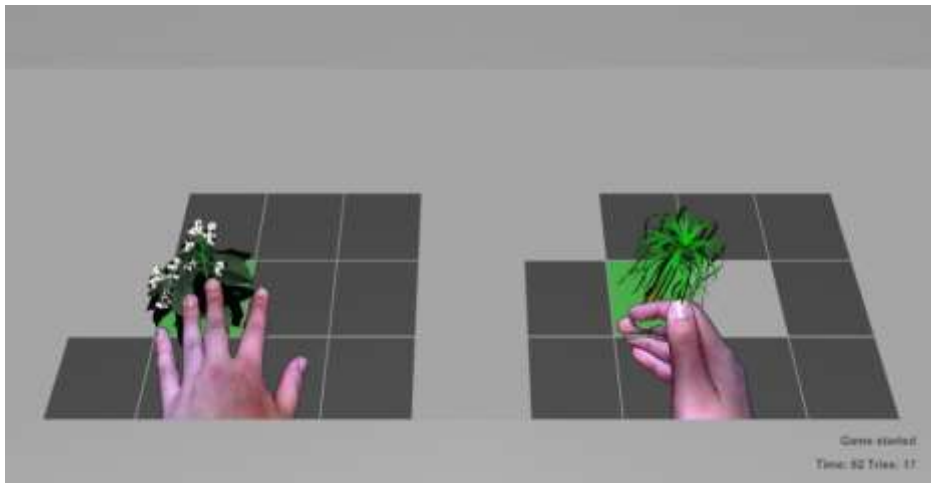


Figure 2. Gameplay of TheraMem for a patient with an impaired right hand.

In this study we use the TheraMem system with patients with stroke to evaluate two capabilities of ART: (a) the augmentation of the visualization of the user's hand within a virtual environment, and (b) manipulation of the displayed hand movement on the screen. The aim is to clinically evaluate the feasibility of using TheraMem for people with chronic (>6 months) stroke in a physiotherapeutic setting.

METHODS

The feasibility of the system for clinical use was evaluated with six patients suffering from motor impairments after a stroke. The evaluation was carried out under the guidance of a physiotherapist with the help of a technical operator who controlled the system and configured the software setting for the individual patients (see Figure 3). This study was preceded by a preliminary study with two subject-matter experts, in which the protocol was tested and fine-tuned. The two experts, of whom one was a trained physiotherapist and the other a postgrad-student with expertise in human-computer interaction, acted as patients but were actively engaged in providing feedback during the session.

System and Clinical Setting

The ART system was placed in a local Physiotherapy Clinic. A secluded room was dedicated for the entire period of the study. The system was placed in a way to allow a quick change between the settings for left and right side impaired people. This was necessary because the physiotherapist was required to sit at the side of the participant's impairment to provide support if required and to get a better picture of how the participant was performing during the exercises.

The operator sat on the left side behind the physiotherapist and the participant. This allowed the operator a good overview and facilitated communication between himself and the

physiotherapist without involving the participant too much. Figure 3 shows a picture of the setting for a participant with a right handed impairment.

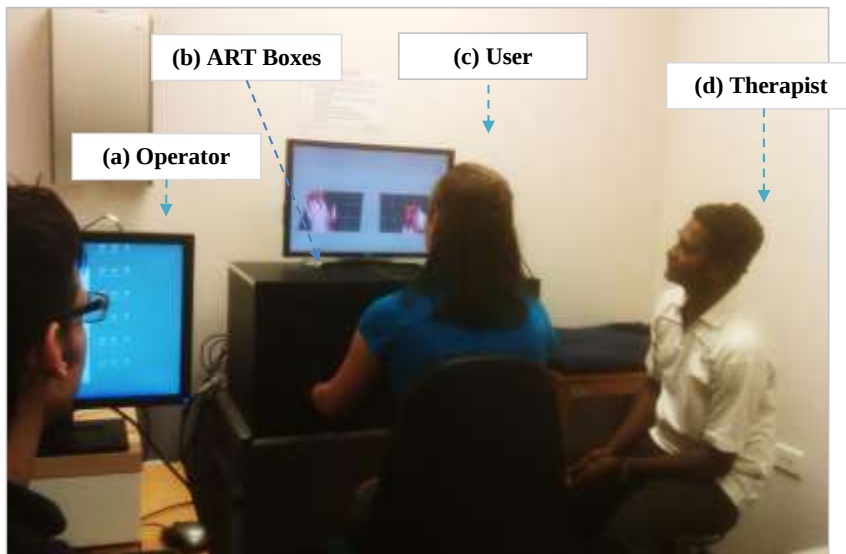


Figure 3. ART's setup for clinical use: (a) Operator screen, (b) Black ART Boxes for hands, (c) User, (d) Physiotherapist.

PARTICIPANTS

Six patients with different types of stroke and varying levels of impairment severity of their upper limbs were recruited from the local stroke club. All patients were > 5 years since the onset of stroke. A detailed description of each participant is provided in Table 1. All participants gave written informed consent. The study was approved by the Regional Health and Disability Ethics Committee (Otago, New Zealand).

In the initial assessment, three patients scored a total of 0 in both the Fugl-Meyer measure (wrist & hand) (6) and the Motor Assessment Scale (upper arm, hand, and advanced hand) (7). The other three patients had moderate ratings in Fugl-Meyer and high ratings in the Motor Assessment Scale.

PROCEDURE AND EXPERIMENTAL DESIGN

Each intervention session lasted for about 60 minutes and included a range of hand exercises using ART. These exercises were chosen by the physiotherapist according to the patient's capabilities and could include: pointing at virtual objects, symmetric movements with mirrored visualization of the hands, following predefined virtual shapes with their impaired hand, or interacting with augmented real objects in the virtual environment. The main part of the exercises however, consisted of the use of TheraMem.

Table 1. Participants' characteristics at admission

Code	Age	Gender	Time since stroke	Cause for stroke and diagnosis	Employment status	Upper limb status
PT0	43 y	F	42 y	Infantile left hemiplegia of unknown cause	Independent, Employed	Moderately impaired, uses affected limb for stabilising
PT1	65 y	M	5.5 y	Right hemiplegia due to CVA	Requires moderate assistance, Retired	Severely impaired. Severe spasticity and deformities involving elbow wrist and hand
PT2	63 y	M	6 y	Left hemiplegia due to CVA	Requires moderate assistance, Retired	Severely impaired. Minimal movements available at the shoulder
PT3	47 y	M	45 y	Right hemiplegia of unknown cause	Independent. Employed	Moderately impaired. Active movement available at all the joints of UE
PT4	53 y	M	15 y	Right hemiplegia due to CVA	Independent. Employed	Moderately impaired. Active movement available at all the joints of UE
PT5	49 Y	M	9 y	Right hemiplegia due to CVA	Independent, stays at home	Moderately impaired. Active movement at shoulder and elbow. Poor control of wrist and fingers

For the TheraMem exercise with patient PT5, in addition to the plant models that the user had to match on both sides, an additional set of 22 virtual models of food were integrated in the system. This was to improve the variety of the exercise between sessions and to respond to the feedback of previous users who expressed their preference for additional virtual models. For each of the four sessions, patient PT5 therefore used TheraMem with a different set of 12 models “under” the tiles that he had to match.

For patients with severe spasticity a brief session of warm-up exercises including manual stretching, ‘weight bearing’ exercises and gentle manual vibration at the shoulder were applied to reduce tightness of the limb.

Each participant was clinically assessed during the first session using the following outcome measures: Fugl-Meyer measure for wrist and hand (6); Motor Assessment Scale (MAS), arm, hand and advanced hand sections (7); Muscle tone assessment with the Modified Ashworth scale (8); and Nine Hole Peg test (9). In addition, an assessment of light touch, pain and proprioception sensation in the affected upper limb was undertaken. The same tests were repeated after the fourth session to evaluate the outcome of the intervention. Tests and treatment were administered by the same physiotherapist.

Patients were also interviewed after each trial with TheraMem about their experience with the exercise and equipment. We explored their motivation and engagement with the technology and what they consider important to change and improve the system settings.

After the last session, patients were interviewed about their general experience with the system as well as asked to suggest possible improvements and applications.

NONCLINICAL MEASURES

The physiotherapist rated the patients’ engagement and performance: the ability to understand the instructions, the execution of movements, the participation in activity, the effort, attitude and the acknowledgement of benefits from the patients on a 6 point scale (with 1 being the lowest).

The operator observed and noted technical details, commented on technical problems and asked patients about their experience during the exposure, for example the perceived difficulties and enjoyment experienced.

RESULTS AND DISCUSSION

A total of 128 exercises were administered of which 56 were exercises with TheraMem. All patients were able to play TheraMem and complete the game at least once, in less than three minutes.

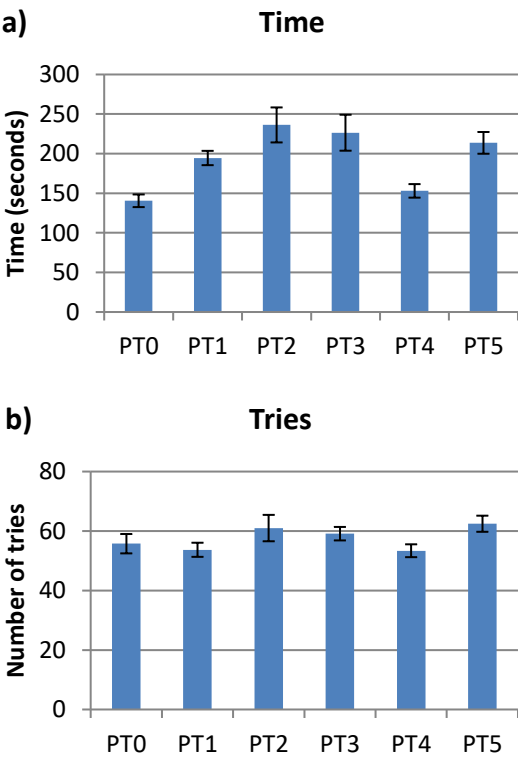


Figure 4. Time (a) and Tries (b) required by patients for completion (Mean +/- Std-error).

The average completion-time (Figure 4a) was 195 seconds (range: 116 to 320 seconds); the two less impaired patients (PT0 and PT4) were able to complete the game faster with an average of 147 seconds (see Figure 4). In an earlier study with healthy participants as published in Regenbrecht, Hoermann et al. (4), an average of 136 was measured; our patients in general were slightly slower. The amount of tries (Figure 4b) that participants required to complete the game was, on average, 57.7 tries (range: 43 to 87), which was similar to the average of 54 tries achieved by healthy participants in the earlier study.

Based on the therapist's ratings on patients' overall engagement, the average was above 5 on a 6 point scale. Accordingly, five of the six patients with an engagement rating of above 5, always found it easy to understand the exercise, demonstrated a very positive attitude towards the exercise, and actively participated in the exercises at their maximal effort, whereas one patient did so only for "most of the time" which was deduced from his engagement rating of only 3.5.

Three support systems were introduced to assist patients with more severe motor impairments in performing the exercise. First was the use of the TheraMem built-in movement amplification, where the performed movement of the participant was amplified so that small movements appeared larger in the game. Second, an elbow-splint was used; it was put on the participants' arm after an initial stretching session and this helped the participant to keep the arm in a relatively straight position. Third, a pointing-device was applied – a wooden stick (tongue depressor), was given to the participants to hold with their impaired hand to facilitate pointing (see Figure 5). This was necessary because three participants were not able to keep their affected hand flat on the floor of the box due to muscle contractures/spasticity and were therefore not able to point with their fingers at a specific tile, but instead had to use their entire hand to select the tile.

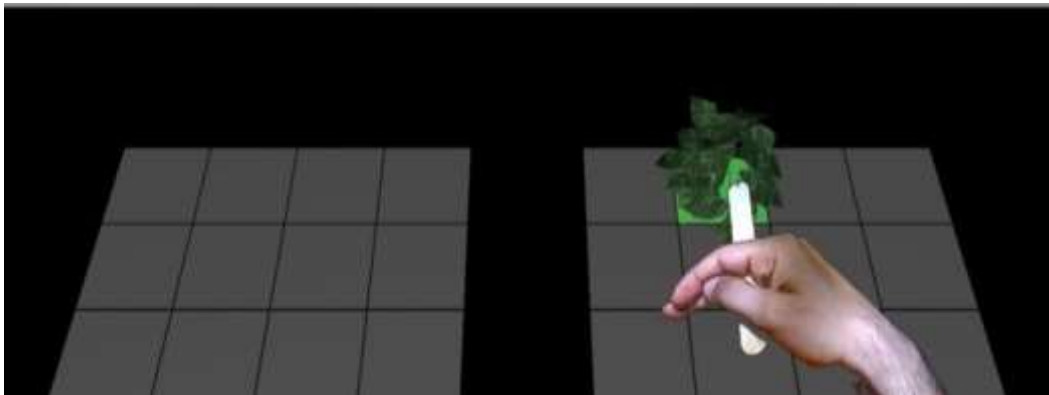


Figure 5. The use of a pointing-device to support the selection of tiles.

Improvements in the clinical outcome measures after the last session were found for three patients. These were the patients with already moderate ratings in the first assessment. One patient (PT0) improved in the Fugl-Meyer measure (hand) from 4 to 9 (/14), in the Motor Assessment Scale (upper arm) from 5 to 6 (/6) and in the Motor Assessment Scale (hand) from 3 to 4 (/6). Patient PT4 improved in the Fugl-Meyer measure (wrist) from 7 to 8 (/10) whereas patient PT5 improved in the Fugl-Meyer measure (hand) from 5 to 6 (/14).

In the final interview all participants expressed the opinion that the ART system has therapeutic potential for the rehabilitation of motor impairments after stroke. They suggested exercises with the ART system be incorporated in the standard set of therapeutic activities that people with stroke are asked to perform during their rehabilitation phase. In addition, participants expressed their interest in continuing this intervention after the last session. This is especially interesting as two of the participants had not undergone physiotherapy for more than a decade.

Patients suggested that the difficulty and variety of the game should be modifiable. The two patients with less motor impairment especially asked for a more challenging gameplay. The current setting with 12 tiles, even though the content was randomized after each trial, was perceived to be decreasingly less challenging for them after they had played the game a couple of times. In addition, these patients asked for more variety of games and suggested the use of more and different sets of 3D models “under” the tiles. This feedback was incorporated in the study with patient PT5 who was evaluated as the last patient and was very well received by him. The enjoyment rating after each exercise with TheraMem for PT5 stayed uniformly high over the four sessions.

All participants felt comfortable and safe in using the system in the clinical environment. In the final interview three patients stated that they could imagine using the system autonomously without the permanent presence of a physiotherapist and one participant even suggested the use of the system at home. The acquisition of an ART system for the local stroke clubs was also suggested.

CONCLUSION

The results of this study showed that the ART system and specifically its TheraMem extension are feasible for use in the rehabilitation of upper limb movement following stroke. Patients were overall, highly engaged and motivated. The addition of extra 3D models to extend TheraMem gameplay variety was a suggestion raised during the sessions with the first five patients. This suggestion was then implemented and successfully evaluated with the sixth patient.

The relatively small improvements for the individual participants in the clinical outcome measures were expected and can be explained by two reasons. Firstly, undergoing only four sessions of intervention might not allow enough time for a significant change. Secondly, all participants were in the chronic phase after stroke and therefore the chances and amount of improvement was limited.

In order to evaluate the clinical outcomes, a larger-scale controlled trial with more sessions and a longer intervention period is recommended. In such a trial we would expect larger improvements especially for people who have had a stroke more recently.

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Chapter 75

COMBINING VIRTUAL REALITY AND A MYOELECTRIC LIMB ORTHOSIS TO RESTORE ACTIVE MOVEMENT AFTER STROKE

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ABSTRACT

We introduce a novel rehabilitation technology for upper limb rehabilitation after stroke that combines a virtual reality training paradigm with a myoelectric robotic limb orthosis. Our rehabilitation system is based on clinical guidelines and is designed to recruit specific motor networks to promote neuronal reorganization. The main hypothesis is that the restoration of active movement facilitates the full engagement of motor control networks during motor training. By using a robotic limb orthosis, we are able to restore active arm movement in severely affected stroke patients. In a pilot evaluation, we have successfully deployed and assessed our system with three chronic stroke patients by means of behavioral data and self-report questionnaires. The results show that our system is able to restore up to 60% of the active movement capability of patients. Further, we show that we can assess the specific contribution of the biceps/triceps movement of the paretic arm in a virtual reality bilateral training task. Questionnaire data show enjoyment and acceptance of the developed rehabilitation system and its VR training task.

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INTRODUCTION

Currently, stroke is one of the main causes of adult disability, and by 2030 it is expected to be one of the main contributors to the burden of disease worldwide (1). An important goal in the management of stroke patients, and in particular in patients with spasticity, involves restoration of normal limb position and ease of passive and active movement execution with the aim of improving functional outcomes such as the ability to carry out activities of daily living (2). This is a very demanding task for trained therapists and especially problematic in patients with low level of motor control and yet aggravated in the presence of spasticity. In fact, 85% of stroke survivors will present a motor deficit contralateral to the location of the brain lesion (3). Additionally, 20-40% will also suffer of increased muscle tone or spasticity, what will further limit their level of independence in the activities of daily living (4,5). The large economical and psychological impacts of stroke on our society, in particular on relatives and public health systems, make it necessary to find alternative and novel approaches to address these issues.

Nowadays, it is well understood that recovery after stroke depends on brain mechanisms that allow undamaged brain areas, such as contralateral or secondary networks, to take over the functions of the damaged areas (6-8). In the chronic stage of stroke, neuronal plasticity is the main contributor to true recovery, being dependent on the size, severity, and location of the lesion (9,10). Therefore, modern rehabilitation approaches should aim at providing an effective way of driving cortical plasticity and recruiting alternative motor areas to achieve functional brain reorganization, while being accessible to the widest range of patients, in particular to those with the poorest prognostic. During the intent to perform a motor action, cortical areas devoted to motor control generate particular activity patterns – reflecting the synchronization and desynchronization of neural activity – known as Sensory Motor Rhythms (11). These activity patterns encode motor control signals that can reach the paretic arm as long as there are remaining cortico-spinal tracts after stroke (12). Control commands effectively transmitted to the limbs can be assessed by measuring electric potentials at the muscles (electromyogram or EMG). Depending on the brain lesion, the amount of motor control and therefore of active movement is compromised.

To overcome this limitation, we propose a hybrid Virtual Reality (VR) and robotic approach for the restoration of correct limb pose and active movement. The objective of our hybrid system is to restore motor control of the upper limbs when active movement is compromised but weak EMG responses are still present. Our technology is able to detect residual EMG activation and, by means of a robotic orthosis, enable motor impaired patients to exercise movement even when active movement is severely compromised. There are data that suggest that the restoration of active movement may play a crucial role in mobilizing cortical plasticity, and therefore in accelerating recovery after stroke. First, although passive movement exercising is able to engage motor networks by means of proprioceptive feedback (13), it has been shown not to be the most effective way of engaging overt execution motor areas (14). Second, the activation of motor related networks does not only depend on the action intent, but also on the type of actions and their completion. It has been shown that both the observation and performance of meaningful goal oriented actions can engage additional networks such as the Mirror Neuron System (MNS), which is also known as the action recognition system (15-17). The discovery of the MNS has allowed the emergence of novel

stroke rehabilitation approaches based on clear neuroscientific hypotheses on brain recovery mechanisms (18-22). In this project, we propose restoration of active movement as a crucial step to fully engage both the motor control networks and the MNS. Therefore, by restoring active movement and engaging patients in a physical training with meaningful goal oriented actions, our hybrid VR robotic system is designed to facilitate true recovery by means of cortical plasticity.

Previous myoelectric driven robotic interventions (23) have been shown to lead to improved Fugl-Meyer scores of the upper extremities (24) and reduced spasticity as assessed by the modified Ashworth score (25). Many control techniques have been explored for myoelectric driven movement assistance such as fuzzy controllers (26) or compliant systems (27,28). In this project, we use a unique wearable and portable orthosis with integrated myoelectric measurement capabilities that restores correct limb position (mPower1000, Myomo Inc., Boston, USA). Further, we believe the combination of the myoelectric orthosis approach with a VR training paradigm is appropriate because of the inherent properties of VR systems for motor rehabilitation. VR approaches allow for a combination of features including: low cost; personalization of training; unsupervised training; goal-oriented actions; adaptability to a broad range of patients; quantifiable outcome measures; extended feedback; and motivation thanks to the use of game elements (29). Our VR training environment builds on previous work (30,31), on training principles that we have shown to be effective in the chronic phase of stroke (32), and to accelerate recovery in the acute phase of stroke (33). Thus, our hybrid system exploits state-of-the-art information and communication technologies, a myoelectric robotic approach, and a neuroscience based rationale to provide a novel personalized rehabilitation training system that addresses the physical sequels and social impact of stroke. The approach presented here puts special emphasis on patients without (or with minimal) active movement capabilities and those with spasticity, enabling them to train active movement (see figure 1).

Our Study

In our approach, we take advantage of the use VR, because it can support requirements for an effective training. VR allows creating fully controlled environments that define training tasks specifically designed to target the individual needs of patients. Additionally, intensive movement training can be supported through motivating tasks that use augmented feedback and reward (29). VR allows not only for the individualization of training and monitoring by physicians, but also enables patients to play a more active role in their rehabilitation process and self-monitor their own improvements. Besides, our VR based rehabilitation system has been integrated in a game like interaction, capitalizing in motivational factors that are essential for recovery (34). Nevertheless, the main novelty of our approach is the combination of an online adaptation during VR training of the level of assistance provided by a robotic limb orthosis with EMG measurement capability (Figure 1). This technology is designed to restore active movement, compensate for fatigue, and optimize training duration, intensity and repetition.

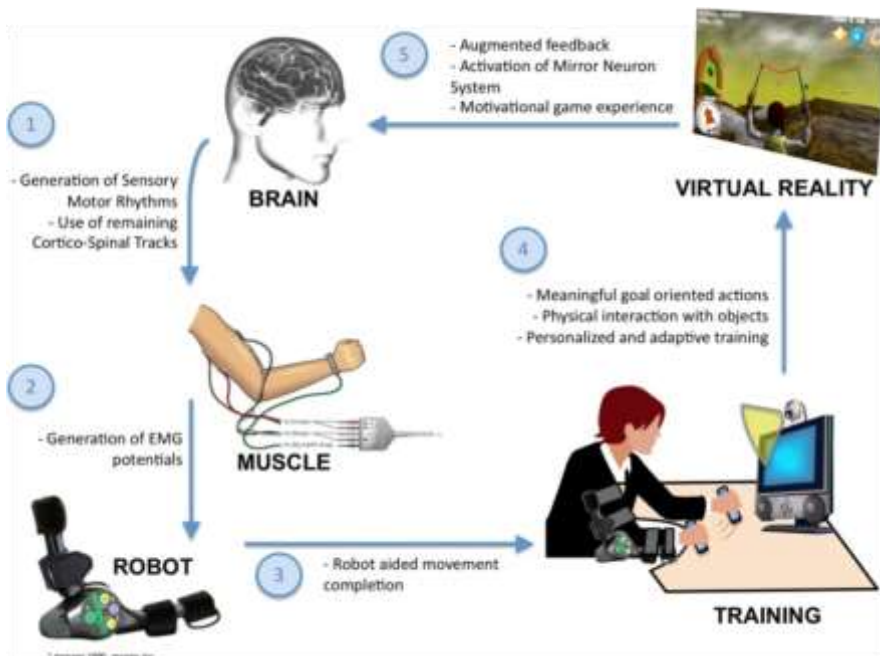


Figure 6. Diagram of the developed virtual reality and robotic limb orthosis training paradigm showing the role of each technological component (numbered from 1 to 5).

Limb Orthosis

The mPower 1000 robotic device (MyomoInc, Boston, USA) is a portable limb orthosis that is controlled through EMG signals that are measured by an on-board data sampler. Two EMG channels and one actuated joint are used to restore active movement based on biceps/triceps EMG activation or relaxation (see figure 1, points 2–3). The mPower assists its user in the completion of arm movements by means of an embedded electric motor that is activated on the detection of biceps and/or triceps EMG activity. The EMG signals are compared to the baseline EMG activity level of the user and an assistive force is executed (either arm extension or contraction) when EMG changes (muscle contraction or relaxation) are detected. This approach makes therapy accessible to patients with residual EMG activation or involuntary and permanent EMG activation, correcting limb position and allowing them to train active contraction/relaxation to gain movement control. The mPower connects to the virtual environment via a virtual serial port over Bluetooth, allowing its remote control from within the training environment. This wireless connection provides information on the orthosis settings, arm position, and EMG readings, and it allows remote adjustment of the level of motor assistance during training from 0 to 100%.

Tracking

The tracking technology used in this project is the ARToolKit (ARToolworks, Inc., Seattle, USA). The ARToolKit is an augmented reality software toolkit that enables tracking

the position (x, y, z) and orientation in space of predefined unique markers by using a webcam as input device. In our system, the ARToolKit was used to track two handles (7 cm diameter $\times$ 12 cm high) tagged with unique visual markers. Thus, an overhead webcam is used to track the position and orientation of the markers, providing the virtual environment with precise information about the position and movement trajectories of the user's hands during training. Users of the system are instructed to grasp and move these handles around a table top in order to interact with the virtual environment (see figure 2, right panel).



Figure 7. Prototype of the myoelectric based interactive system for rehabilitation. Left panel: An adaptive training in the form of a game defines the training parameters for a bimanual coordination motor task. The training offers augmented feedback on performance, sustains motivation, and automatically modifies the level of motor assistance offered by the limb orthosis. Right panel: The different components of the system (robotic device, tracking setup, and training game task) while being used by a stroke patient.

Virtual Environment

The virtual environment and training task are based on the Neurorehabilitation Training Toolkit (NTT), which is freely accessible at [http:// neurorehabilitation.m-iti.org/NTT](http://neurorehabilitation.m-iti.org/NTT). The NTT is a virtual training environment developed with the open-source game engine Panda3D (www.panda.org) that was designed following neuroscientific and therapeutic guidelines for stroke rehabilitation, such as relevance of training to ADLs, neuronal mechanisms of recovery, narrative, personalization or individualization, augmented feedback, and engagement (30) for a detailed description of the training rational). In essence, the training task is a game experience consisting of a bimanual coordination task that uses upper limb motor actions as control signals. Bimanual upper limb training was selected because it has been shown to enhance excitability of cortical motor networks and lead to improved functional outcomes (35,36). The bimanual motor actions are mapped onto the actions of an avatar that controls a glider in the virtual environment, i.e., the physical arm movements of

the user are used to control the steering direction of a virtual glider (see figure 2, left panel). Feedback on performance and on-screen information is extensively used to inform the user on the immediate game goals and motor actions to be performed, as well as a reward mechanism. The goal of the game is to gather the largest number possible of collectable items in the virtual environment. Two types of collectable objects are present – easy (balloons) and difficult (stars) – that are accumulated to an on-screen score to provide feedback on performance. In addition, the amount of arm movement measured by the limb orthosis is also provided as a visual score. All tracking and training data are logged as a text file for later analysis.

Table 1. Patients’ demographics

Age	47	63	50
Sex	Male	Male	Male
Stroke type	Hemorrhagic	Ischemic	Ischemic
Stroke location	left	left	right
Handedness	Right	Right	Right

Pilot Evaluation

The objective of this pilot study was to assess the acceptance and usability of the system, as well as the impact of the level of assistance of the limb orthosis on task performance and overall arm movement. We evaluated the system with 3 chronic stroke survivors (47–63 years old; > 6 months post-stroke) in a laboratory setting at the University of Pittsburgh (see table 1). All subjects had a very low level of control of their paretic arm but were able to generate voluntary EMG activation, and hence able to drive the robotic orthosis. All subjects used the robotic orthosis in biceps mode (only controlled by biceps EMG activity) and used the system for a single training session of approximately 20 minutes. During the training session, the level of assistance of the orthosis was randomly changed between 40% and 90% every time a virtual item was collected. After the training session, subjects were asked to report on their experience by answering a questionnaire about enjoyment, engagement and usability rated using a Likert scale from 1 to 5. All subjects gave their informed consent to participate in this study.

FINDINGS

This is a unique system that not only engages users in a game-like VR training experience, but also makes use of a myoelectric capable orthosis to restore active movement. However, the effectiveness of the orthosis assistance in movement restoration and how to optimally integrate it in an interactive training experience need to be studied before any longitudinal deployment. For these reasons, we performed a number of experiments in which we exposed three stroke survivors to single training sessions of the combined VR and myoelectric limb orthosis paradigm. The integrated system allows us to simultaneously

measure both the movement of the arm end effector (tracked by a marker on the handle) and the specific movement of the biceps as measured by the orthosis (see figure 2, right panel). Training data were recorded synchronously with tracking data as well as the limb orthosis settings.

The analysis of the multimodal data revealed a linear effect of the level of assistance of the limb orthosis with the amount of biceps movement as measured by the system in deg/s (see figure 3, left panel). The existence of this linear relationship between level of assistance and movement execution is an important feature of the system because it will enable us to integrate, into the VR training environment itself, straight forward statistical modeling techniques to automatically adjust the level of assistance depending on the characteristics of each user. This will allow us to improve the level of motor control or compensate for fatigue or loss of force during training. Moreover, the system provides us with additional valuable data, allowing us to quantify the particular contribution of the biceps/triceps movement to overall movement (Figure 3, middle panel). In our experiment we could assess that the movement of the elbow joint (measured by the limb orthosis) showed a low correlation coefficient with that of the end effector (37). This reveals a low contribution of the elbow joint, and therefore a low biceps/triceps contribution, to the bimanual control task defined in our training. This correlation indicates that possible compensatory strategies were used during training. Further, our VR training system allows us assess and compare differences between paretic and non-paretic arms. This enables us to monitor recovery over time using the non-paretic arm as reference. Of particular interest is the comparison of movement capability of the paretic and non-paretic arms when the orthosis assistance is enabled. During our pilot experiment we have quantified the impact of the active orthosis on the overall movement of the arm and we verified that the myoelectric orthosis was able to restore the paretic arm movement to about 60% of the non-paretic arm in patient 3. These results are yet more remarkable when compared to the absence of assistance, in which case the overall movement of the paretic arm was below 30% of that of the non-paretic one (see figure 3, right panel).

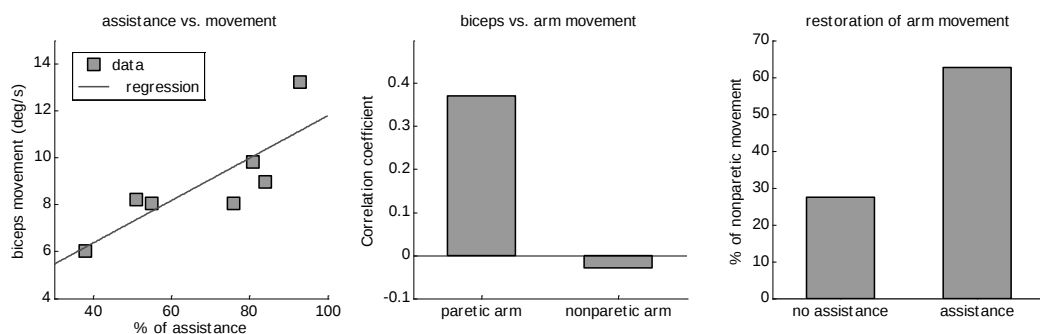


Figure 8. Effect of the myoelectric limb orthosis during the virtual training task. Left panel: Effect of the level of assistance of the limb orthosis on the amount of biceps movement. Middle panel: Quantification of the contribution of the biceps movement to the overall arm movement, computed as the correlation value of the biceps and arm movements during training. Right panel: Restoration of arm movement. % of arm movement of the paretic arm as compared to the non-paretic arm in presence and absence of robotic assistance. Data from patient 3.

Questionnaire data revealed a good acceptance of the system, the most positive aspects being: fun [4.3], entertaining [4], and willingness to use it as regular motor training [4.6]. Subjects reported that the system was easy to understand [3.6] but also considered it a challenging training task [1.6].

DISCUSSION

Here we presented a novel hybrid system that integrates a VR training and a myoelectric limb orthosis. This system is an extension of the Neurorehabilitation Training Toolkit (NTT) that aims at restoring arm movement in severely affected stroke patients by integrating an EMG driven portable robotic limb orthosis. This system is specifically designed to be used by patients without or with minimal active movement capabilities, i.e., those with the poorest prognostic, enabling them to train active movement. The robotic orthosis is combined with a gaming training environment that is based on clinical guidelines and designed to recruit the MNS and to promote neuronal reorganization. By using a robotic limb orthosis, we are able to restore active arm movement in severely affected stroke patients. We hypothesize that the restoration of active movement facilitates the full engagement of motor control networks during motor training.

In this first pilot experiment, we have successfully deployed and tested our bio-hybrid VR interactive rehabilitation system with three chronic stroke patients. The system was evaluated by means of quantitative behavioral data – acquired by the system itself – and self-report questionnaires. Initial results show that our system is capable of online adjusting the assistance level provided by the orthosis, and that the level of assistance has a linear effect on the overall arm movement. We showed that the myoelectric orthosis is able to restore up to 60% of the active movement capability. Although encouraging, these results require further investigation to better understand how the level of assistance relates to improvements in motor control and also to the overall recovery process of the paretic arm in a longitudinal intervention. Another of the strengths of the presented approach is that our technology allows assessing the individual contribution of the biceps/triceps movement to the overall bilateral training task. We have shown that we can objectively assess and monitor the active contribution of the elbow joint to overall arm movement as well as detect and quantify the presence of compensatory strategies. Questionnaire data reveal a high level of acceptance of the system and its VR training task, although it was found to be challenging. This is an expectable result because in this experimental protocol patients with no or very low active movement were exposed to varying levels of orthosis assistance, including low levels of assistance. This effect will be minimized in the future by using an algorithmic solution to automatically adjust the level of assistance to each patient, thus maximizing the outcome of training. The long-term impact of these technologies will be assessed in a randomized controlled trial in the inpatient rehabilitation unit (RHB) of the Hospital of Funchal.

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Chapter 76

CHILDHOOD VASCULITIC STROKE

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ABSTRACT

Arteriopathy is the leading cause of childhood arterial ischemic stroke, but the pathophysiology is poorly understood. Vascular inflammation is a frequently considered, but rarely proven mechanism. This chapter considers the pathophysiology of primary and secondary vasculitides, and their role in pediatric stroke. Pathologically-proven small vessel central nervous system vasculitis is an inflammatory disease model with treatments that improve outcome while primary large vessel arteriopathy, which has many features suggestive of inflammation, and typically lacks definitive histologic proof of vasculitis. Potentially useful biomarkers, advances in neuroimaging, and the pathophysiological approach to childhood vasculitic stroke will be discussed along with unanswered clinical questions and controversies.

OVERVIEW

Stroke is defined as focal cerebral injury secondary to occlusion or rupture of a cerebral blood vessel. Ischemic stroke can occur when the occlusion is present in an artery (arterial ischemic stroke [AIS]) or vein (cerebral sinus venous thrombosis [CSVT]). Hemorrhagic stroke results from a rupture of a cerebral blood vessel. The estimated incidence of childhood AIS is 0.6 to 7.9 per 100,000 while that of hemorrhagic stroke is 0.4 to 13.0 per 100,000 [1]. Childhood stroke causes significant lifelong morbidity, mortality, and economic burden [2-4]. Although prothrombotic, cardiac and hematologic risk factors play a role in pediatric stroke, disorders of the cerebral blood vessels themselves or vasculopathy are the leading mechanism

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of both their cause and recurrence [5-7]. An incomplete understanding of pathophysiology of childhood vasculopathy has precluded the development of targeted therapy and prevention strategies. A recent large international study of childhood AIS [5] suggested that inflammatory mechanisms were a leading cause of arteriopathy, implicating vasculitis and the role of anti-inflammatory medication therapy. However, such suggestions are highly controversial and not yet part of expert consensus guidelines for stroke management in children [8, 9]. Childhood primary angiitis of the central nervous system (cPACNS) represents the prototypical syndromes of pediatric vasculitic stroke and is a recognized category of inflammatory brain diseases of childhood [10]; and an important cause of pediatric AIS. Pediatric cerebrovascular disorders and CNS vasculitis are considered elsewhere [11, 12].

VASCULAR PATTERNS OF STROKE

Arterial Involvement

Patterns of stroke are identified by the region of brain supplied by the affected vessel. AIS patterns are mainly classified as large- or small-vessel infarcts (Figure 1).

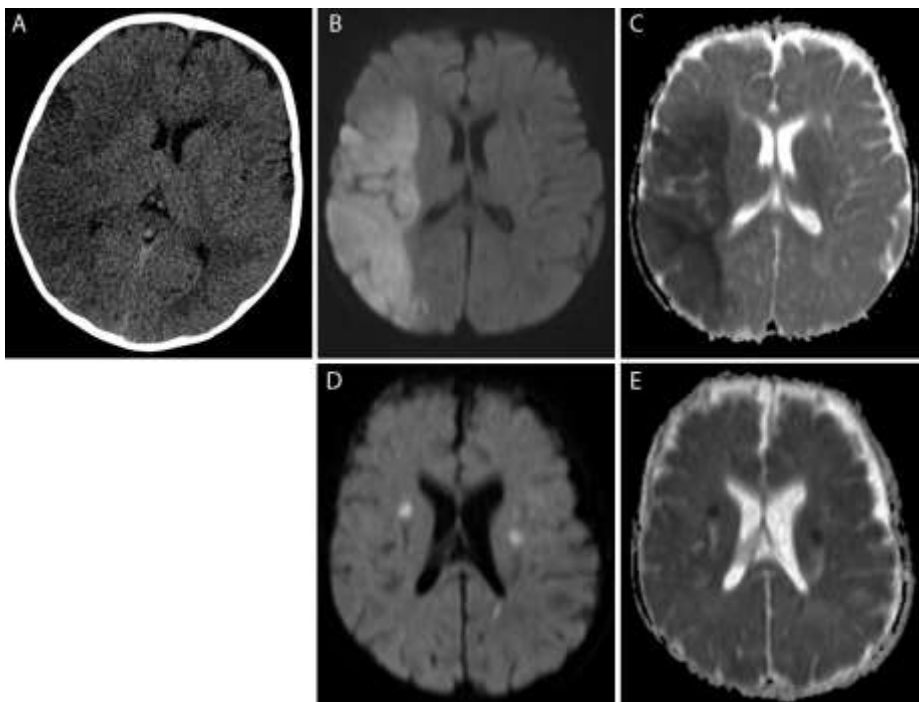


Figure 1. Vascular stroke patterns. A – C. Large vessel right middle cerebral artery (MCA) territory ischemic stroke. D- E. Small vessel ischemic infarcts. Hypodensity on axial CT (A) in the right MCA territory indicative of acute ischemic changes. Hyperintensity on axial DWI (B) and hypointensity on axial ADC (C), consistent with restricted diffusion in the right MCA territory indicative of an acute arterial ischemic stroke in this territory. Bilateral, punctate hyperintense DWI (D) and hypointense on ADC (E) lesions represent small vessel ischemic infarcts.

Large vessel infarcts typically result in wedge-shaped parenchymal lesions that occur secondary to occlusion of branches of the major arteries of the circle of Willis. Small vessel diseases result in smaller, often multifocal or diffuse parenchymal infarcts with highly variable imaging appearances. The vessel size and distribution of infarct helps determine the etiology of the stroke. Symptoms and signs are dependent on the area of cerebral damage. Large vessel infarcts usually present with sudden onset focal neurological deficits such as contralateral hemiparesis when the middle cerebral artery (MCA) is occluded. In contrast, strokes in small vessel territories more often present with subacute, non-localizing neurological complaints such as headache, behavioral changes, seizures, or cognitive decline [13-15].

Venous Involvement

Cerebral sinus venous thrombosis is identified by thrombus in the cerebral venous system. Unlike arterial strokes, this does not always lead to parenchymal injury. If the increased venous blood pressure exceeds arterial pressure, insufficient blood supply leads to focal ischemia. Secondary hemorrhage often occurs due to venous back pressure in ischemic lesions.

In fact, purely ischemic infarcts were described in only 13% of 160 pediatric CSVT cases compared to hemorrhagic infarcts in 30 - 40% [16, 17]. The clinical features of CSVT can be subtle, non-specific, and diffuse. Patients can present with signs and symptoms of raised intracranial pressure such as protracted headaches, papilledema and diplopia, seizures, or decreased level of consciousness [18].

STROKE CLASSIFICATION

Adult stroke etiologies are often classified according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria which divided stroke subtypes into large-artery atherosclerotic, cardioembolic, small vessel lacunar, other determined and undetermined etiology [19]. Despite common use in adult stroke care and research, applicability of TOAST criteria appears limited in young stroke patients where many etiologies are classified as other or undetermined [20].

In childhood AIS, where non-atherosclerotic arteriopathy predominates, reliance on TOAST classification is inadequate to further the understanding of disease [5-7]. Thus, a new system with a more comprehensive classification of pediatric stroke etiologies is currently being developed. With a focus on arteriopathy, the Childhood AIS Standardized Classification and Diagnostic Evaluation (CASCADE) attempts to address the unique causes of stroke in children [21].

This is important step forward because it highlights the importance of considering vascular inflammatory mechanisms. CASCADE specifies diagnostic categories that consider the possibility of vasculitis. As elaborated below, categories include small-vessel arteriopathy of childhood, unilateral focal cerebral arteriopathy (FCA) of childhood, transient cerebral

arteriopathy (TCA), bilateral cerebral arteriopathy of childhood, post-varicella angiopathy (PVA), aortic/cervical arteriopathy, cardioembolic, and multifactorial etiologies [21].

NOSOLOGY AND CLASSIFICATION OF VASCULITIS

Vasculitis of the central nervous system is divided into primary and secondary causes. Secondary causes occur in the context of other systemic conditions such as systemic vasculitides. Vasculitis is defined as inflammation of blood vessel walls for at least some time during the course of the disease and can affect arteries and veins of varying caliber. The Revised 2012 Chapel Hill Consensus Conferences (CHCC) [22] provides a useful nosology for the classification of vasculitis according to the caliber of vessels involved, vessel type, and the associated clinical and pathologic features. Large vessel vasculitis, typified by giant cell arteritis (GCA) and Takayasu arteritis (TAK), affects the aorta, its major branches and analogous veins. The prototypical forms of medium vessel vasculitis include polyarteritis nodosa (PAN) and Kawasaki disease (KD), which involves main visceral arteries, veins and initial branches. The category of small vessel vasculitis recognizes involvement of intraparenchymal arteries, arterioles, capillaries, veins and venules, with a disease mechanism related to anti-neutrophil cytoplasmic antibody (ANCA) and circulating immune complexes (IC). The category of ANCA-associated vasculitis (AAV) includes granulomatosis with polyangiitis (GPA), eosinophilic granulomatosis with polyangiitis (EGPA), and microscopic polyangiitis (MPA), while vasculitic disorders associated with circulating IC include IgA vasculitis-associated Henoch-Schönlein purpura (IgAV/HSP), cryoglobulinemic vasculitis (CV), and C1q antibody-associated hypocomplementemia urticarial vasculitis (C1q/HUV). Vasculitis without a predominant vessel size and caliber, respectively from small to large, involving arteries, veins and capillaries, comprises the category of variable vessel vasculitis, characteristic of Behçet disease (BD) and Cogan syndrome (CS). There is a category of vasculitis associated with systemic disorders such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), sarcoidosis, and Sjogren syndrome. A category of vasculitis associated with an infectious process is described by a prefix for the presumed etiopathogenic agent. The category of single-organ vasculitis (SOV) is exemplified by adult and childhood PACNS.

Although applicable to pediatric patients, this nosology [22] was not specifically designed for this population. Accordingly, the European League against Rheumatism (EULAR) and the Pediatric Rheumatology European Society (PReS) [23] developed consensus criteria for the classification of childhood vasculitis designated cPAN, cGPA, and cMPA for the childhood forms of the respective adult disorders, with others including, TAK, KD, IgAV/HSP were unabbreviated because of their common pediatric occurrence. Childhood PACNS and primary CNS vasculitis (PCNSV) are equivalent terms for a spectrum of disease subtypes, still incompletely classified and distinguished by vessel size, angiographic and pathological findings, and the presence or absence of long-term progression [24, 25]; they are further divided into large-medium and small-vessel forms. Table 1 summarizes the potential inflammatory and non-inflammatory etiologies of childhood CNS vasculopathy.

Table 1. Etiology of CNS Vasculopathy in Children*

Primary CNS vasculitis	Large-Medium Vessel Progressive Non-progressive FCA TCA PVA Small vessel
Systemic Vasculitis	Large Vessel Vasculitis Takayasu arteritis Medium Vessel Vasculitis Polyarteritis Nodosa Kawasaki Disease Small Vessel Vasculitis ANCA-Associated Vasculitis (GPA, EGPA, MPA) Immune-Complex Vasculitis (CV, IgAV, HUV(C1q) Variable Vessel Vasculitis (BD, CS)
Other Vasculitides	Sjogren syndrome Systemic lupus erythematosus Behçet syndrome
Post- and Para-infectious vasculitis	Bacterial <i>Mycobacterium tuberculosis</i> <i>Mycoplasma pneumonia</i> <i>Streptococcus pneumonia</i> Viral HIV/AIDS Varicella zoster Parvovirus B19 Influenza A Enterovirus Spirochete <i>Borrelia Burgdorferi</i>
Systemic Inflammatory Disease	Inflammatory bowel disease Neurosarcoidosis
Non-Inflammatory Arteriopathy	Moyamoya syndrome Fibromuscular dysplasia Dissection Radiation arteriopathy Hemoglobinopathy (Sickle cell disease) CADASIL Fabry disease Reversible cerebral vasoconstriction syndrome
Drug-Induced Arteriopathy	Cocaine Amphetamines

*Adapted from [23, 42, 43, 51]; and Gowdie P, Twilt M, Benseler SM. Primary and secondary central nervous system vasculitis. *J Child Neurol* 2012; 27: 1448-1459. Abbreviations: FCA, focal cerebral arteriopathy; TCA, transient cerebral arteriopathy; PVA, post-varicella arteriopathy; ANCA, anti-neutrophil cytoplasm antibody; GPA, granulomatosis with polyangiitis; EGPA, eosinophilic granulomatosis with polyangiitis; MPA, microscopic polyangiitis; CV: cryoglobulinemic vasculitis; IgAV, IgA vasculitis; HUV C1q, hypocomplementemia urticarial vasculitis associated with C1q antibodies; BD: Behçet disease; CS, Cogan syndrome; HIV, human immunodeficiency virus; AIDS, acquired immune deficiency virus syndrome; CADASIL, Cerebral autosomal dominant arteriopathy with subcortical Infarcts.

PRIMARY VASCULITIDES

Large Vessel Vasculitis

Systemic large vessel vasculitis leads to inflammatory stenosis of the aorta and its proximal branches. Takayasu arteritis (TAK) is the prototypical childhood disorder diagnosed in the presence of one of the following criteria including, decreased peripheral pulses and/or claudication, blood pressure difference of > 10 mmHg between arms, bruits over the aorta or its branches, or hypertension [23]. Stroke occurrence has been noted in 17% of 230 reported children with TAK between 1994 and 2008 [26]. There is a risk of focal AIS from thromboembolism as well as hypoperfusion-induced brain infarction secondary to proximal arterial stenosis. While the pediatric presentation of TAK includes hypertension similar to adults [27], affected children more commonly manifest fever, weight loss, abdominal pain, and headaches [26].

Medium Vessel Vasculitis

Two classic medium vessel vasculitides PAN and KD may manifest neurological involvement including a small risk of cerebrovascular disease. The diagnosis of cPAN requires evidence of systemic inflammatory disease with evidence of necrotizing vasculitis or angiographic abnormalities of medium- to small-sized arteries plus one of five criteria including, skin involvement, myalgia, hypertension, peripheral neuropathy, and renal involvement [28]. Neurological manifestations are seen in up to 50% of patients but consist mostly of peripheral nervous system involvement [29, 30]. In a survey of patients with PAN, 16% of 110 pediatric patients (but one third of those with systemic PAN) had neurological symptoms. Four of 63 patients with systemic PAN had cerebral aneurysms [31] and cases of intracerebral hemorrhage have also been reported in the literature [32].

The diagnosis of KD requires the presence of fever for at least five days plus four of the following criteria including, desquamation of extremities or perineum, polymorphous exanthema, bilateral conjunctiva injection, injection of oral or pharyngeal mucosa, and cervical lymphadenopathy [23]. The disease affects the coronary arteries and stroke as a complication has only rarely been reported, predominantly large-vessel cardioembolic stroke but also cerebral micro-hemorrhages. [33-35] Evaluation of 24 patients with KD using magnetic resonance imaging (MRI) revealed only one cryptogenic cardioembolic posterior inferior cerebellar artery (PICA) infarct [36]. Risk is potentially higher in patients with ventricular dysfunction secondary to myocardial infarction or arrhythmia. Thus, although a rare occurrence, stroke should be considered in children with signs or histories of medium vessel vasculitis and neurological symptoms, particularly acute focal neurological deficits.

Small Vessel Vasculitis

GPA, classified as an AAV affecting small vessels, is recognized by the presence of two of nasal-oral inflammation, abnormal chest x-ray, urinalysis, and granulomatous

inflammation on tissue biopsy [23]. Neurologically, it is most often associated with contiguous extension of granulomas leading to pachymeningitis while reports of cerebral vasculitis or AIS are rare. Similarly, children with EGPA rarely present with AIS [37]. IgAV/HSP is a common disorder of early childhood characterized by nonthrombocytopenic purpura, arthralgia, nephritis, and abdominal pain [29, 30]. Stroke types associated with IgAV/HSP include hemorrhagic and arterial ischemic strokes as well as sinus venous thrombosis [29, 30]. However, pediatric stroke is rare in IgAV/HSP, described in only 1-8% of patients [29, 38, 39].

Variable Vessel Vasculitis

Behçet syndrome is a rare primary systemic vasculitis of childhood characterized by mucocutaneous ulcers and uveitis. CNS involvement includes idiopathic intracranial hypertension and meningoencephalitis which occur in up to 50% of patients [29]. Cerebrovascular disease has been reported in 30% of adults. Occurrence in children is recognized in isolated case reports but the true incidence is unknown and likely low. Venous sinus thrombosis may be most common but arterial infarcts and aneurysms are also described [40].

CNS Vasculitis

Isolated central nervous system vasculitis in children has been referred to in the literature as cPACNS. Specific subtypes are distinguished based on vessel size, angiographic and pathological findings, and the presence or absence of long-term progression [24, 25]. Large-medium vessel vasculitis is divided into progressive and non-progressive types [24, 25, 41], the latter syndrome being highly comparable to other childhood cerebral arteriopathies such as TCA and FCA. Current classifications remain incomplete and controversial, as discussed here and elsewhere [24, 25, 42] but provides a practical, clinically relevant framework. It should be noted that the following discussion is based on evidence supporting a vasculitic mechanism for large-medium cPACNS but that definitive diagnostic testing does not exist and the acceptance of this mechanism varies widely within the pediatric stroke community.

Large-Medium Vessel Type

Most large/medium vessel primary CNS vasculitis in children is non-progressive and unilateral. As such, it bears marked similarity to other cerebral arteriopathies causing stroke in otherwise healthy children, namely TCA and FCA. It is possible they represent the same disease or closely related diseases within a spectrum [41, 43]. The following features are shared across these syndromes and are used in support of the clinical diagnosis [6, 21, 25, 42]:

- (1) Location: Unilateral arteriopathy of the large vessels of the anterior circulation, typically the distal internal carotid artery (ICA) and proximal segments of the MCA and anterior cerebral artery (ACA).
- (2) Arteriopathy characteristics: Unique angiographic appearance with unilateral focal and segmental stenosis often with contiguous alternating areas of stenosis and dilatation with a “banding” or “striated” appearance. More definitive features of other arteriopathies such as dissection or Moyamoya are absent (see differential diagnosis below).
- (3) Time course: A dynamic nature with early serial vascular imaging often demonstrating a fluctuating course of arterial changes on a scale of days to weeks.
- (4) Resolution: A monophasic illness when followed over the longer term with follow up imaging after 6 months confirming no progression, and variable resolution of arterial changes.

A representative case example of this syndrome is presented in Figure 2. Ironically, the most common syndrome of AIS in school-aged children is also the least understood. Definitive evidence of an inflammatory pathophysiology is lacking in most cases, supporting the consideration of alternative diagnostic terminologies. The diagnosis of TCA requires angiography within 3 months of stroke demonstrating unilateral focal or segmental stenosis or occlusion of the distal internal carotid or proximal MCA and ACA and lack of progression at 6 months [42, 44, 45]. Moreover, FCA shares the same features as TCA [6, 21] but facilitates earlier diagnosis, respecting the inability to exclude progression until 6 months later; PVA discussed below shares many of these features. Therefore, while a legitimate diagnostic entity of its own [41, 43, 46], the non-progressive variety of large-vessel cPACNS shares many features with other childhood cerebral arteriopathies, the spectrum of which should be considered in the evaluation and management of affected children.

Bilateral large vessel arteriopathy without collaterals is a distinct, less common disease classification within CASCADE [21]. Research to date has been limited but suggests large vessel cPACNS may be bilateral in up to 20% of children [41, 47]. Whether this represents a more widespread version of the more typical disease (akin to optic neuritis), a marker of progressive large vessel cPACNS [41, 45], or falls within a spectrum of other bilateral arteriopathies such as fibromuscular dysplasia or Moyamoya disease [45] remains to be determined. Progressive large-medium vessel cPACNS also appears to be uncommon and is more likely to present with long-standing, non-focal, diffuse symptoms such as neurocognitive dysfunction, headaches, and seizures. Imaging may reveal multifocal, bilateral parenchymal lesions on MRI, and multiple, bilateral stenoses of the distal vessels on angiography [24, 41]. Ante mortem tissue diagnosis is not possible due to involvement of larger intracranial arteries. The result is reliance on neuroimaging, specifically angiography, which continues to improve but cannot definitively implicate inflammatory pathophysiology or immediately identify a progressive from non-progressive prognosis. Long-term surveillance and outcome studies are required to better understand the natural history and predictors of recurrent forms of large-medium vessel cPACNS.

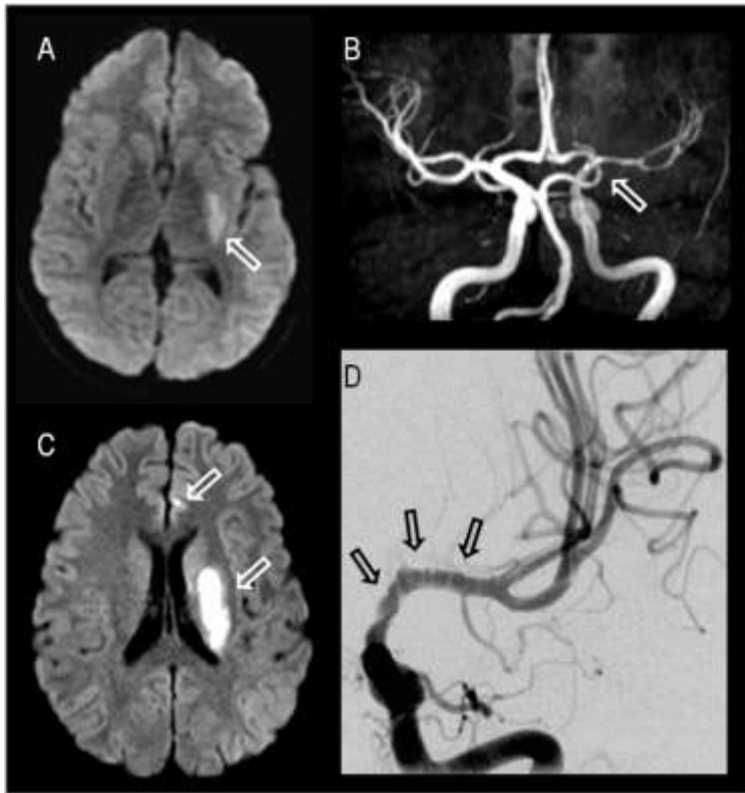


Figure 2. Focal cerebral arteriopathy and AIS in a 15-year-old boy with acute right hemiplegia and hemianesthesia. (A) Diffusion MRI on day 1 shows faint restricted diffusion limited to the posterior putamen and internal capsule. (B) MRA shows reduced ICA and MCA flow with irregular narrowing of left MCA (arrow). (C) Repeat diffusion MRI on day 11 shows new restricted diffusion extending throughout the corona radiata and small cortical areas within the MCA and ACA territories (arrows). (D) Conventional angiography (left ICA injection) demonstrates severe irregularity of the distal ICA and proximal MCA with alternating “bands” of narrowing. The ACA is now occluded.

Small Vessel Type

Primary vasculitis of the small cerebral blood vessels is better defined than those described above, involving large vessels. Unlike large vessel disease, definitive evidence of vessel wall inflammation is often obtained by brain biopsy, allowing pathological confirmation of vasculitis [15, 48]. Patients may present with focal symptoms but are more likely to develop subacute, non-localizing neurological complaints such as headache, behavioral changes, seizures, school failure or cognitive decline [13-15]. Strokes, when they occur, do not conform to large vessel territories and ischemic lesions can be highly variable in character and distribution [15, 48]. Hemorrhagic lesions may also occur although the incidence is probably <10% [49]. Neuroimaging is far less specific compared to the large vessel diseases described above. Non-invasive arterial imaging employing computed tomography angiography (CTA) and magnetic resonance angiography (MRA) is typically normal while parenchymal MRI can range from normal to diffusely abnormal with a wide array of lesion characteristics [15]. Conventional angiography is also often negative and small vessel cPACNS may also be referred to as angiography-negative vasculitis [14, 15, 43, 50]. Diagnostic criteria proposed by Calabrese and colleagues [46] for adult PACNS have been

applied to children [41, 51]. These include: (1) an acquired neurologic deficit that remains unexplained after thorough evaluation, (2) either classic angiographic or histopathologic features of angitis within the CNS, and (3) no evidence of systemic vasculitis or any other condition to which the angiographic or pathologic features could be attributed [46]. In the face of less severely symptomatic children when angiography is negative and brain biopsy is deferred, the clinical diagnoses of “probable” small vessel cPACNS may be considered.

SECONDARY VASCULITIDES

Connective Tissue Disorders

Neuropsychiatric manifestations in SLE may occur as a direct consequence of the disease, such as antibody-mediated neuronal dysfunction, or its treatment. Vascular mechanisms may also occur including complement activated vascular inflammation, Libman-Sacks endocarditis, or thrombosis due to hypercoagulable states associated with high titers of lupus anticoagulant and phospholipid antibodies [52]. The risk of AIS, CSVT, and intracranial hemorrhage are all increased in SLE. Patients most commonly present with angiogram-negative small vessel vasculitis or sinus venous thrombosis [52]. Of 185 pediatric patients with SLE reviewed retrospectively, 64 (34.6%) had neuropsychiatric symptoms: 1 (0.5%) had transient ischemic attack (TIA), 18 (9.7%) had ischemic stroke, 4 (2.1%) had venous thrombosis, and 2 (1.1%) had intracranial hemorrhage [53]. Another study found cerebrovascular disease to be present in a total of 24% of their retrospective cohort [52]. Cardioembolic stroke secondary to Libman-Sacks, nonbacterial endocarditis has rarely been reported in children [54]. Sjogren syndrome is an autoimmune disease rarely encountered in children [55]. Case reports of adolescents with Sjogren syndrome and stroke have been reported [56, 57], as did one presenting with Moyamoya syndrome. A study comparing patients of all ages found cerebrovascular disease was less common in Sjogren syndrome compared to SLE despite comparable incidence of neuropsychiatric symptoms [58].

Infection

An association of pediatric stroke with infection has long been suspected [59], and the associated pathophysiology may be explained by direct infection of cerebral arteries or a “triggering” effect leading to inflammatory cerebral arteriopathy that is para- or post-infectious. The most direct evidence is described in children with stroke associated with bacterial meningitis, in which the pathophysiology is presumed to result from inflammation of perforating vessels in the subarachnoid space that traverse infected meninges [60, 61]. Large artery involvement has also been reported in association with bacterial meningitis [60, 61]. A late-onset, large vessel arteriopathy, beginning during the recovery phases of bacterial meningitis is associated with poor outcome [62, 63]. The incidence of this infective arteriopathy may be underestimated, reported in 37% of consecutive adults undergoing conventional angiography after bacterial meningitis [64]. The diagnosis of meningitis-related arteriopathy is relatively easy with clear evidence of bacterial meningitis. However, other

infectious mechanisms may be more occult, the timelines less clear, and their associations with stroke less definitive.

Although the association of large vessel cerebral arteriopathy in children with inflammation is unclear, there are many similarities between large/medium cPACNS PVA. An association was demonstrated between *Varicella zoster virus* (VZV) infection was suggested in case-control [65] and cohort studies [66] of childhood AIS. Focal cerebral arteriopathy associated with recent VZV infection has also been implied by case reports [67-74]. Cerebrospinal fluid (CSF) pleocytosis [72, 73, 75] and VZV antigens [67, 70, 71, 73] have occasionally been demonstrated in patients with stroke and recent VZV infection. Herpes zoster ophthalmicus associated with arteriopathy and stroke has been described in both adults [76-78] and children [79-81]. Tissue analysis from biopsy [82] and postmortem studied patients with PVA [83] revealed vasculitis with lymphocytic infiltration and VZV-positive immunohistochemistry with antigen deposition in the smooth muscle layer of the vessel wall, suggested both direct infiltration and immune-mediated inflammation.

The clinical presentation, neuroimaging, and natural history of PVA are indistinguishable from TCA except for the history of VZV infection within the preceding 12 months [42]. The course is monophasic with subsequent stabilization or improvement of arteriopathy over months [84]. Consistent with proximal MCA involvement, infarct patterns typically involve lenticulostriate territories resulting primarily in deep basal ganglia infarcts [44, 45]. Proximal segments of the ICA and ACA may also be involved as seen in TCA, FCA and cPACNS. Therefore both direct and indirect parainfectious mechanisms may be responsible for some vasculitis-associated strokes in children.

Other infectious agents are less commonly associated with arteriopathic stroke in children (Table 1); and this list is illustrative rather than exhaustive. Fusiform aneurysms, focal arteriopathy, and stroke occur in children with human immunodeficiency virus type 1 (HIV1) [85-87] and vasculitis has been shown on autopsy in children with the acquired immune deficiency syndrome (AIDS) [88]. Isolated reports have associated neuroborreliosis, [89-91] parvovirus B19, [92, 93], influenza A [94], Enterovirus [95], and *Mycoplasma pneumoniae* [96-98] with focal cerebral arteriopathy and stroke in the pediatric population. It is important to note that isolated case reports do not prove causation [25].

Rather than specific infectious pathogens, para- or post-infectious mechanisms in general have been implicated in childhood cerebral arteriopathy pathogenesis and increased stroke risk. Population-based studies have demonstrated associations between recent preceding infections and arterial strokes in children [6]. The association between acute infections and increased risk of vascular events is also recognized in the adult literature [99-101]. The Vascular Effects of Infection in Pediatric Stroke (VIPS) study is an international research endeavor designed to test the hypothesis that infection predisposes children to arteriopathy, AIS, and stroke recurrence [102].

DIAGNOSTIC WORK UP

The diagnostic work up of childhood stroke due to arteriopathy is summarized in Table 2.

Table 2. Diagnostic Algorithm of Childhood Stroke Due to CNS Vasculitis*

History	Neurological review of systems (focal, headaches, seizures, other) Past Medical History Family History Infectious exposures/Risk factors for stroke History of skin, ophthalmologic, joint involvement, recurrent fevers
Physical Examination	Vital signs, peripheral pulses General and neurological examinations Rheumatologic assessment Neuropsychological assessment
Laboratory Investigation	<i>Markers of Inflammation:</i> CBC, CRP, ESR, IgG, C3, vWF <i>Prothrombotic Markers:</i> Protein S, C, ATIII, fibrinogen, plasminogen, homocysteine; factor V Leiden, PT gene mutations, lupus anticoagulant. <i>Autoantibodies:</i> ANA, ENA, dsDNA, ANCA, aPL, ACL (depending on the differential diagnosis: NMDAR, VGKC, GAD, GABA) <i>Infectious serology:</i> <i>B. burgdorferi</i> , VDRL, HIV, VZV
Lumbar CSF Analysis	OP, cell count, protein, glucose, IgG, OCB Cryptococcal antigen, VDRL, viral encephalitis register paired serum and CSF <i>B. burgdorferi</i> and VZV serology bacterial, fungal, TB cultures specific autoantibodies depending on the differential diagnosis
Neuroimaging	CT/CTA MRI/MRA with gadolinium and vessel wall imaging Cerebral angiography Brain SPECT PET/CT Doppler ultrasound
Tissue Biopsies	Brain and overlying meninges Skin, nerve, muscle as indicated

*Adapted from [115 and 128]. Abbreviations: CBC, complete blood count; ATIII, CRP: C-reactive protein, ESR, erythrocyte sedimentation rate, C3, C3 complement; ATIII, anti-thrombin III; VWF, Von Willebrand factor; ELISA, enzyme linked immunosorbent assay; CSF, cerebrospinal fluid; PT, prothrombin; LAC, lupus anticoagulant; ANA, antinuclear antibody; ENA, extractable nuclear antibody; dsDNA, double-stranded DNA antibody; aPL and ACL, anti-phospholipid and anti-cardiolipin antibodies; NMDAR, N-methyl-D-aspartate receptor; VGKC, voltage-gated calcium channel, GABA, gamma-aminobutyric acid; VZV, varicella-zoster virus; CT/CTA, computed tomography and angiography; MRI and MRA, magnetic resonance imaging and angiography; SPECT, single photon emission computed tomography; PET/CT, positron emission tomography and computed tomography; IgG, immunoglobulin G; VDRL, Venereal disease research laboratory; TB, tuberculosis; OCB, oligoclonal bands; GAD, glutamate decarboxylase; C3, complement component 3.

Differential Diagnosis

Numerous non-inflammatory conditions must be considered in the differential diagnosis of CNS vasculitis. One mimic of large vessel CNS disease described above is arterial dissection, an important cause of stroke which may extend intracranially more often in

children [5, 103]. The importance of differentiating FCA from intracranial dissection was recently highlighted by the description of four patients with classic FCA presentations but pathologic and wall imaging evidence of intracranial dissection [104]. Additional well defined, non-inflammatory childhood arteriopathies include Moyamoya disease or syndrome [105], post-radiation vasculopathy [106], and other congenital arteriopathies [42]. Reversible cerebral vasoconstriction syndrome (RCVS; Call-Fleming) has rarely been described in children [107]. Fibromuscular dysplasia (FMD) is different in children from adult FMD, and associated with arteriopathic stroke, systemic arteriopathy and hypertension [108].

Several conditions may also mimic small vessel vasculitis in children. Given the diverse nature of both the clinical and imaging findings, broad categories must be considered including demyelinating diseases such as acute disseminated encephalomyelitis, multiple sclerosis, toxin exposures, inborn errors of metabolism, hypertensive crisis, neoplasia, notably CNS lymphoma, and others. A difficult clinical distinction is made between small vessel vasculitis and an increasing number of autoimmune encephalitides. Patients with autoimmune encephalitis present with symptoms of headaches, personality changes, and other neurological findings with non-specific lesions on imaging, as described in small vessel vasculitis, however, the distinguishing features are normal cerebral angiography and different serum biomarkers and/or brain biopsy pathology [109]. Skip lesions reported in primary granulomatous angiitis of the CNS can further confound the diagnostic picture [110], however full-thickness, lesional biopsies minimize the possibility of false-negative results [43, 111].

Serologic Studies

Most children with cPACNS do not manifest systemic inflammatory markers as the disease process is isolated to the CNS. Acute phase reactants such as ESR, CRP, and vWF are often normal or only mildly elevated. Antinuclear antibody titers are also only variably elevated [13, 15, 112]. One single-centre, retrospective study of 62 children with cPACNS [41] found elevations in ESR in 51% of patients, CRP in 74%, IgG in 35%, anticardiolipin antibodies in 44%, without ANCA. Elevations of inflammatory markers were not predictive of outcome, progression, or disease type. Another single-centre cohort of patients with cPACNS [113] found that 65% of 39 patients with both small and large-medium vessel vasculitis had elevated vWF titers that correlated with disease activity. The findings of elevated levels of tumor necrosis factor (TNF)- α , interleukin (IL)-2, IL-6, and IL-8 in childhood AIS [114] suggests their potential usefulness in the laboratory evaluation of cPACNS. Secondary CNS vasculitis is generally suggested after a detailed history and physical examination. The presence of elevated non-specific inflammatory markers should direct the laboratory investigation to serologically-specific disease markers, particularly those that would dictate treatment such as SLE [52].

Cerebrospinal Fluid Analysis

CSF analysis is essential for excluding crucial considerations in the differential diagnosis such as infection [115]. Although often normal [13, 15, 48, 116], elevated opening pressure,

pleocytosis, and raised CSF protein have been described in both large and small vessel cPACNS [14, 15, 112]. Abnormal CSF was described in 39% (9/23) patients in a single-centre, retrospective study [41] of 62 children with cPACNS. Findings included non-specific or lymphocyte-predominant pleocytosis in 32% and the same proportion with elevated protein. Oligoclonal bands were absent. CSF markers were not associated with outcome or progression of disease. However, the numbers were too small to distinguish differences between subtypes of vasculitis.

Isolated case reports of children with PVA have shown mild CSF pleocytosis. [80] The detection of CSF anti-VZV IgG antibodies is possibly more sensitive than VZV PCR testing with some [117] suggesting the diagnosis can be excluded when CSF is negative for both. The use of an antibody index (AI) to better determine significant elevations of VZV antibodies in CSF has been proposed as more sensitive [118]. With the exact role of VZV in childhood arteriopathy yet to be determined, these findings and other isolated cases of infection-associated arteriopathy [89-91, 95] suggest that additional methods to define CSF biomarkers are required [102].

Neuroimaging

The diagnosis of a child with acute focal neurological deficits is stroke until proven otherwise. It requires urgent imaging in the form of CT or MRI depending on availability of scan and stability of the patient. Given the high incidence of arteriopathy in childhood stroke, angiography should be included in the initial imaging protocol [8]. For children being evaluated for subacute or progressive neurological changes such as headaches, cognitive or behavioral changes, MRI is the modality of choice.

Advanced cerebral vascular imaging modalities have increased the sensitivity of detecting and defining the unique features of childhood arteriopathies. CT, MR and conventional angiography are used to confirm arteriopathy in most children with large vessel arteriopathy. Inflammation is considered if stenosis, narrowing, or occlusion is seen without evidence of dissection and when associated with luminal irregularities or other indications of arterial wall disease. However, it is important to note that there are no validated imaging biomarkers of inflammation in childhood stroke [25]. CTA and MRA produce excellent images of first and second order cerebral arteries but resolution of smaller vessels is limited.

The diagnosis of large-medium vessel vasculitis according to the criteria of Calabrese and colleagues [46] requires demonstration of the angiographic findings of arteritis if brain biopsy is not feasible. Conventional angiography is the preferred modality for demonstrating specific vascular abnormalities. Banding or striae are key findings in large-medium vessel vasculitis (Figure 2D). Similarly, alternating areas of stenosis and dilatation in distal arterial beds are seen in small vessel cPACNS [24, 51]. Sensitivity is only moderate for predominantly distal, small vessel disease [47]. Conventional angiography which is very safe in the pediatric population [119] should be considered in children with AIS.

Less invasive vascular imaging modalities are preferable for serial surveillance of fluctuating disease or syndromes with high recurrence risk. When comparing MRA to conventional angiography, sensitivity and specificity for known angiographic abnormalities identified on MRA were 70% and 98% respectively in one study [47]. MRI and MRA have been evaluated in single centre cohort studies. All patients with abnormal conventional

angiography were found to have abnormalities on parenchymal MR imaging but only 71% had MRA abnormalities [120]. Conventional angiography is clearly more sensitive than MRA but angiography-negative cases are common in children [47]. MRA remains an essential tool for initial screening and serial follow-up over time.

Despite the low specificity of MRI for the detection of parenchymal lesions in small vessel vasculitis, a combined approach with other clinical markers may increase the diagnostic yield. Angiogram-negative cases often have parenchymal MRI abnormalities [41, 50, 120] including, supratentorial, asymmetrical, anterior circulation lesions of the white matter or deep gray structures [41, 120]. Imaging predictors of progressive vasculitis are multifocal, bilateral, and gray matter lesions, combined with multiple, bilateral, or distal vessel stenoses [41]. Recently introduced vessel wall imaging techniques including high resolution anatomical and blood sensitive sequences have been developed to help distinguish arterial pathology in adult CNS vasculitis [121-125]. Such approaches are not yet well described in children but carry the excellent potential to address the pathophysiology of CNS vasculitis. Case reports of FCA suggesting reversible wall enhancement have been published [126]. Recently described normal cerebral arterial wall enhancement may assist in defining abnormal patterns of stroke in children [127]. Further studies in this area could provide additional non-invasive imaging biomarkers of inflammation or other processes, as well as, the evolution of disease over time and response to treatment.

Diagnostic Tissue Pathology

Pathological confirmation of vasculitis is the gold standard of diagnosis, however obtaining required tissue may be difficult or unfeasible. Brain biopsy may be feasible and even diagnostic, however in small vessel cPACNS, MRA and cerebral angiography are usually normal. In children, a non-granulomatous predominantly T-cell lymphocytic vasculitis of small vessels is typically found in lesional and non-lesional biopsies of angiography-negative cPACNS. Occasional macrophages, polymorphonuclear cells, and eosinophils may also be present. Involved cerebral vessels in leptomeninges, cortical gray and subcortical white matter demonstrate intramural and perivascular inflammatory cell infiltrates [14, 15, 50, 112] with surrounding reactive changes [14]. Granulomatous angiitis of the CNS is distinctly uncommon in children [116]. Full-thickness combined leptomeningeal and cortical biopsy, ideally localized to an area of image-based disease is preferred when possible although non-lesional “blind” biopsies can incur significant diagnostic potential [43, 111]. Systemic or secondary vasculitides may offer additional tissue diagnostic options such as skin, muscle, or nerve biopsy in PAN, renal biopsy in GPA and IgAV [23], or salivary gland biopsy in Sjogren syndrome [55]. A more unified and systematic approach for obtaining and analyzing brain biopsy tissue is required and being developed.

TREATMENT

Recognizing the real possibility of vasculitis in children with stroke, published consensus guidelines [8] acknowledge consideration of anti-inflammatory or immunosuppressive

interventions but offer little direction. The tenets of childhood stroke treatment include acute neuroprotective care and antithrombotic therapies including antiplatelet or anticoagulation strategies, the latter particularly indicated in CSVT or cardiogenic AIS. A decision to couple such treatments with an anti-inflammatory intervention when primary or secondary cerebral vasculitis is suspected is more difficult. Perhaps the only clear indications for immunomodulation are strokes associated with confirmed systemic inflammatory disease or pathologically proven small vessel cPACNS. Published protocols for cPACNS [15, 43, 50] include six months of oral corticosteroids coupled with monthly pulses of intravenous cyclophosphamide followed by maintenance therapy employing azathioprine or other low dose maintenance therapy for an additional eighteen months. Alternative approaches may consider initial high-dose oral corticosteroids for three months [43, 128] and methotrexate and/or mycophenolate mofetil for long-term maintenance therapy [13, 51]. So while precise agents, dosing, and protocols remain to be determined, immunomodulation is clearly indicated in certain childhood vasculitic stroke syndromes.

Immunomodulation for large-medium cPACNS is more controversial. Early recurrence risk is high and children often progress in both arteriopathy severity and symptomatic ischemia in the days and weeks following diagnosis. With the ability of antithrombotic medications to prevent early recurrence unproven and likely modest, many will consider introduction of anti-inflammatory treatments, particularly in high risk patients. Steroids are most often used with reasonable extrapolation from historical adult stroke trials suggesting relative safety. Ease of administration, rapid onset of action, and even positive effects on cerebral perfusion pressure merit consideration. Time courses are not well established but may parallel the natural history of cPACNS, TCA, or PCA where disease activity is expected to diminish over months. Patients with PVA have also been treated with short courses of intravenous methylprednisolone and two weeks of acyclovir [67, 129, 130], although some have questioned the benefit of such an approach in VZV-associated stroke [80]. Others [66, 84] reason that acute antiviral treatment is unnecessary as acyclovir only suppresses active viral replication while an effect on autoimmune reactions by dormant virus seem unlikely [118].

The management of secondary CNS vasculitis focuses on AIS in the context of the associated systemic inflammatory disorder. Combination therapy employing corticosteroids and cyclophosphamide is appropriate for stroke in association with IgA, as is intravenous high-dose methylprednisolone followed by maintenance oral corticosteroid tapered over 3 months for CSVT in BD [38]. Stroke prevention in TAK focuses on prevention of progression of disease in the aorta and its branches employing disease modifying agents and surgical interventions with grafting and stenting if necessary [131-133].

Rehabilitation should be addressed based upon a model of early multidisciplinary assessment and intervention to prevent secondary complications of AIS and reduce disability and financial burden according to published guidelines [134]. This typically entails a traditional therapy program of exercises to promote muscle strength and development, the integration of assistive devices such as orthotics and splints. [135] Management of associated comorbidities includes anticonvulsants for symptomatic epilepsy, behavioral management notably for attention deficit hyperactivity disorder, and neuropsychological testing and support for educational and vocational needs to optimize outcomes.

CONCLUSIONS

The most common cause of stroke in healthy children is arteriopathy. The expansion of knowledge in the recognition, diagnosis, and treatment of pediatric vasculitic cerebral arteriopathy witnessed in the past decade carries important implications for current approaches to diagnosis and treatment while defining a clear need for improvements in both.

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Chapter 77

STROKE DUE TO VASCULITIS IN ADULTS

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ABSTRACT

The vasculitides are diseases characterized by inflammation of blood vessels and inflammatory leukocytes in vessel walls. There is an increased propensity for ischemic stroke as a result of compromise in vessel lumina and distal tissue ischemia, with hemorrhagic stroke and aneurysmal bleeding due to loss of vessel integrity.

INTRODUCTION

The vasculitides are diseases characterized by inflammation of blood vessels and inflammatory leukocytes in their walls. There is an increased propensity for ischemic stroke as a result of compromise of in vessel lumina and distal tissue ischemia, and hemorrhagic stroke and aneurysmal bleeding due to loss of vessel integrity. The 2012 Revised Chapel Hill Consensus Conference (CHCC) [1] nomenclature provides systemic nosology and a categorization of primary and secondary vasculitides. Central nervous system (CNS) involvement and stroke both occur in the single-organ vasculitic (SOV) syndrome primary CNS vasculitis (PCNSV) [2], as well as, due to limited or multiorgan manifestations of primary systemic vasculitides [1].

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PRIMARY CNS VASCULITIS

With several proposed diagnostic schemes for PCNSV over the years [3-5], the combination of clinical and histopathological findings derived from cerebral angiography and leptomeningeal and brain biopsy remains the recommended method of definite diagnosis [6]. This approach facilitates the identification of clinicopathologic subtypes such as those with persistent focal deficits and stroke [2] or intracranial hemorrhage [7] from the reversible cerebral vasoconstriction syndrome (RCVS) [8] that may mimic true vasculitis but does not require cytotoxic therapy.

Historically, Younger and colleagues [3], who described four patients with granulomatous angiitis of the brain histopathologically manifesting necrotizing vasculitis of arteries and veins of varying caliber in conjunction with giant cells and epithelioid cells, noted non-focal symptoms of headache and mental change, followed by focal weakness, seizures, and fatal coma in all four patients. Focal weakness with a stroke onset was noted in two (Patient 1 and 3) of the four patients, Patient 1 of whom had onset of lethargy, focal ptosis and hemiparesis had predominant anterior (ACA), middle (MCA), posterior cerebral artery (PCA) and basilar artery involvement at postmortem examination. Patient 3, who presented with hemiparesis, ataxia, and dysarthria, was later found to have predominant small leptomeningeal artery and vein involvement. Altogether, 15% of 78 patients overall had focal weakness with stroke onset compared to 42% who presented with focal deficits during the course of the illness.

In the same year, 1988, Calabrese and Mallek [4] described eight patients with classic angiographic or histopathologic features of PCNSV or so called primary angiitis of the CNS (PACNS), and reviewed the literature of 40 literature cases. One (patient 2) [9] who presented with the clinical diagnosis of isolated angiitis of the CNS (IACNS) defined by classical angiographic features, had a clinical onset of transient weakness that progressed to fixed hemiparesis, nonfluent aphasia, alexia, and agraphia with later stabilization after combination prednisone and cyclophosphamide therapy.

Birnbaum and colleagues [5] proposed changes to the criteria of Calabrese and Mallek [4] to include a definite diagnosis of PCNSV only after tissue biopsy confirmation, noting strokes or persistent deficits in less than 20% of patients at onset, and with only about 28% of symptoms overall related to large cerebral vessel involvement. Salvarani and co-workers [2] noted that persistent neurological deficit or stroke and headache were the commonest initial symptoms affecting 68% of 101 patients so studied with PCNSV defined by diagnostic criteria similar to those suggested earlier [4]. Cerebral infarction was noted on magnetic resonance imaging (MRI) of the brain in 53% of patients that were multiple in appearances in 85%, bilateral in 83%, and involved the cortex and subcortex in 63%. Overall, their appearance suggested large-artery, branch-artery or small-artery distributions. Intracranial hemorrhage was noted in 8% of patients. Gadolinium enhancement was found in one-third of patients, a quarter of which demonstrated gadolinium enhancement of the meninges.

Montefort and co-workers [10] described a 57-year-old man with brain-biopsy confirmed PCNSV and a 2-month history of right arm weakness and numbness superimposed on a history of left-sided headache and intermittent visual field loss. Brain MRI showed evidence of cortical infarction across more than one vascular territory with signal changes consistent with hemorrhage in the left basal ganglia and parieto-occipital lobe. There was angiographic

evidence of irregular narrowing and aneurysmal dilatation of the left terminal carotid artery which stabilized without new areas of new infarction after treatment with intravenous pulsed cyclophosphamide and corticosteroids followed by azathioprine. The neuroimaging features of this patient are shown in Figures 1 to 4, as well as, in a similarly affected patient in Figures 5 and 6.

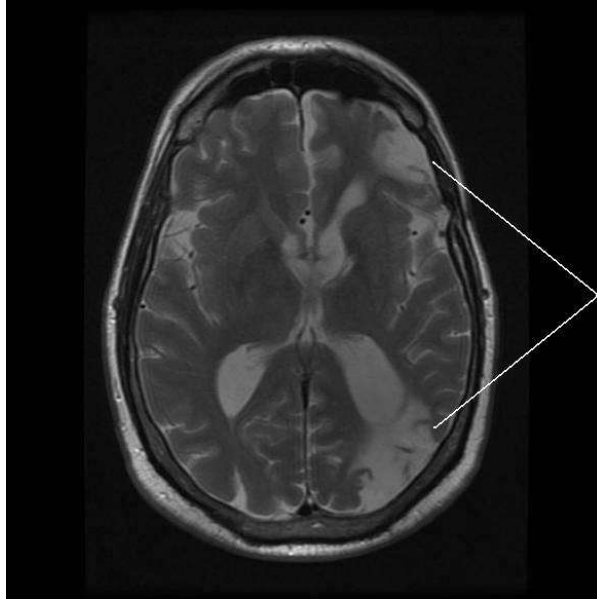


Figure 1. Primary CNS vasculitis. T₂-weighted MRI of the brain demonstrates left frontal and parieto-occipital lobe areas of infarction. Reproduced with permission from [10].

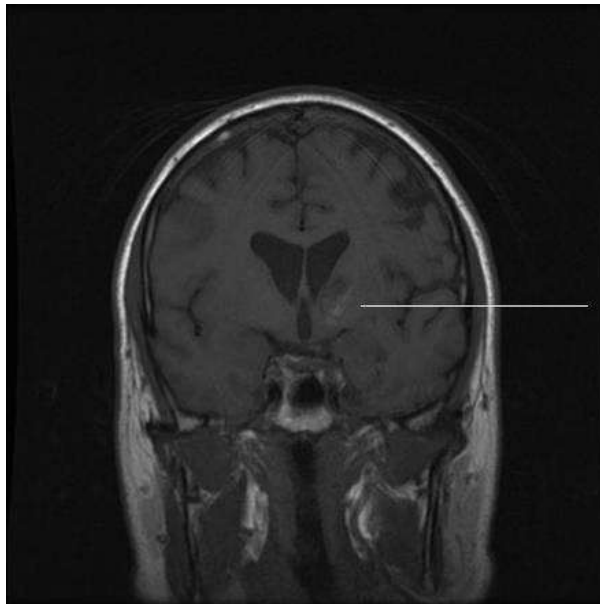


Figure 2. Primary CNS vasculitis. T₁-weighted MRI of the brain showed left basal ganglia haemorrhage. Reproduced with permission from [10].

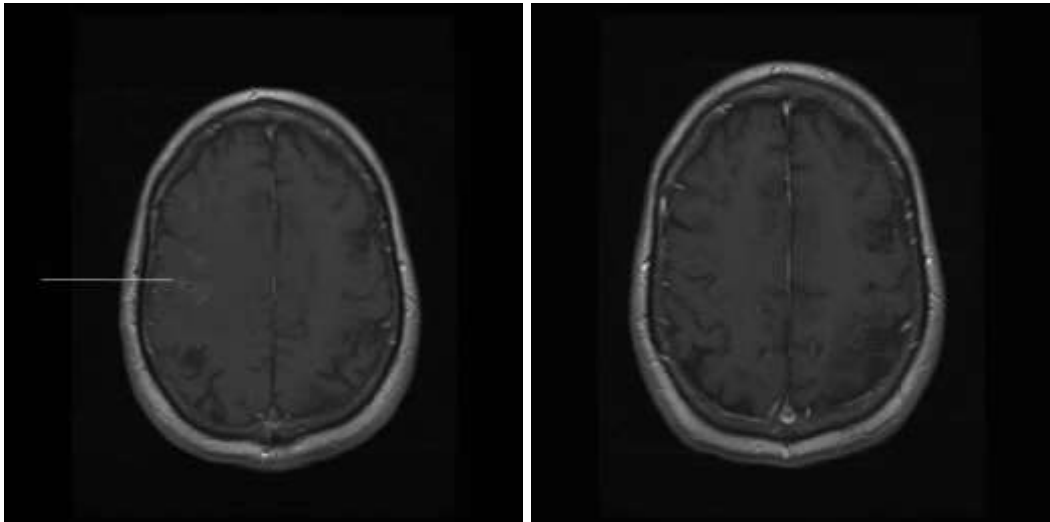


Figure 3. Primary CNS vasculitis. T1-weighted MRI of the brain demonstrates gyral enhancement after intravenous administration of gadolinium before treatment (A), that resolves after immunosuppressant therapy (B). Reproduced with permission from [10].

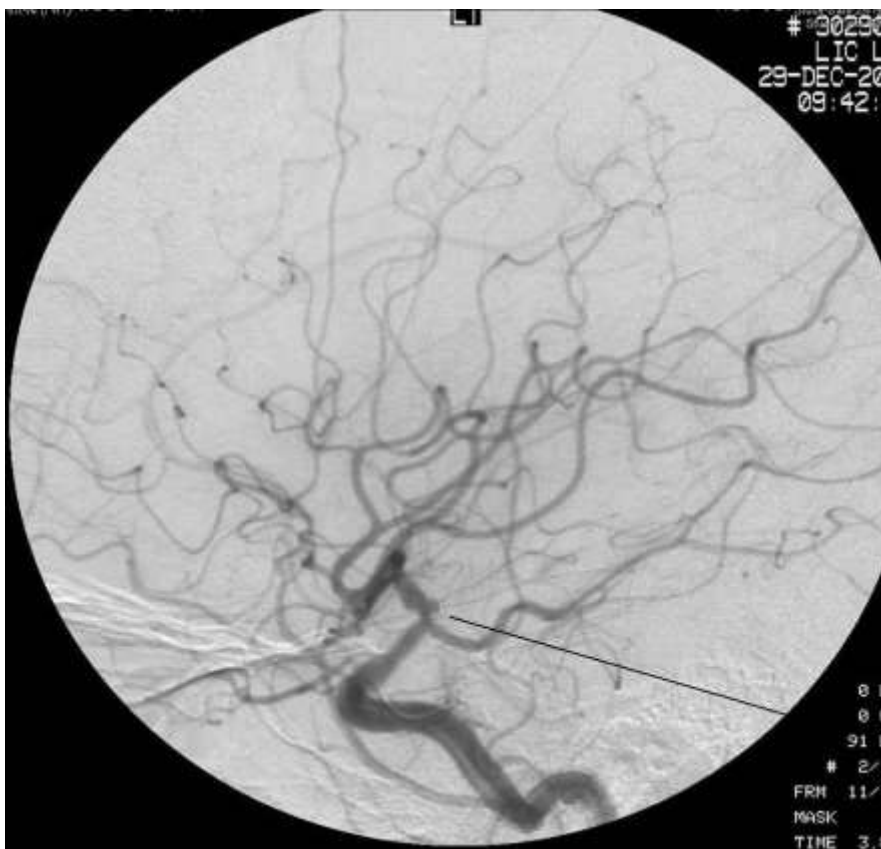


Figure 4. Primary CNS vasculitis. Cerebral angiography shows a sessile internal carotid artery aneurysm. Reproduced with permission from reference 10.

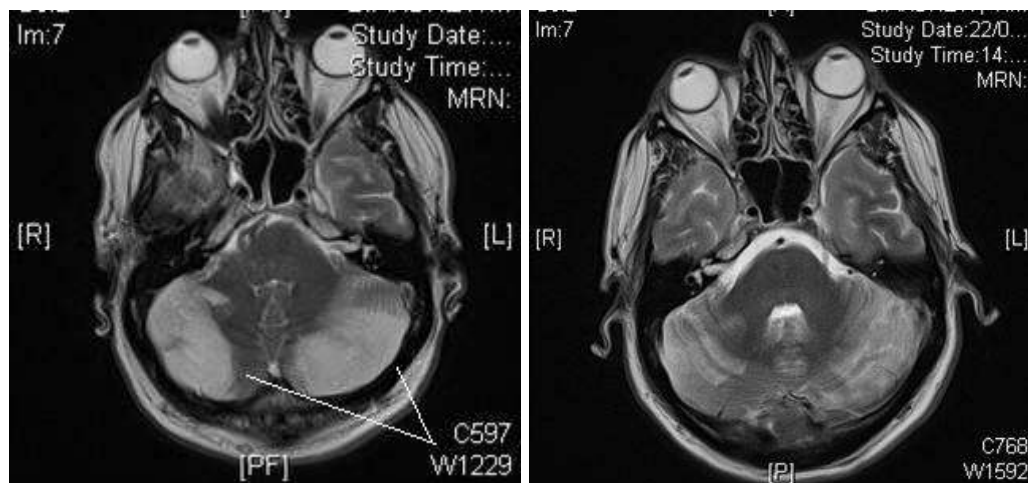


Figure 5. Primary CNS vasculitis. T₂-weighted MRI of the brain shows bilateral cerebellar hemispheric infarcts and abnormal signal hyperintensities before treatment (A) that resolve after immunosuppressant.

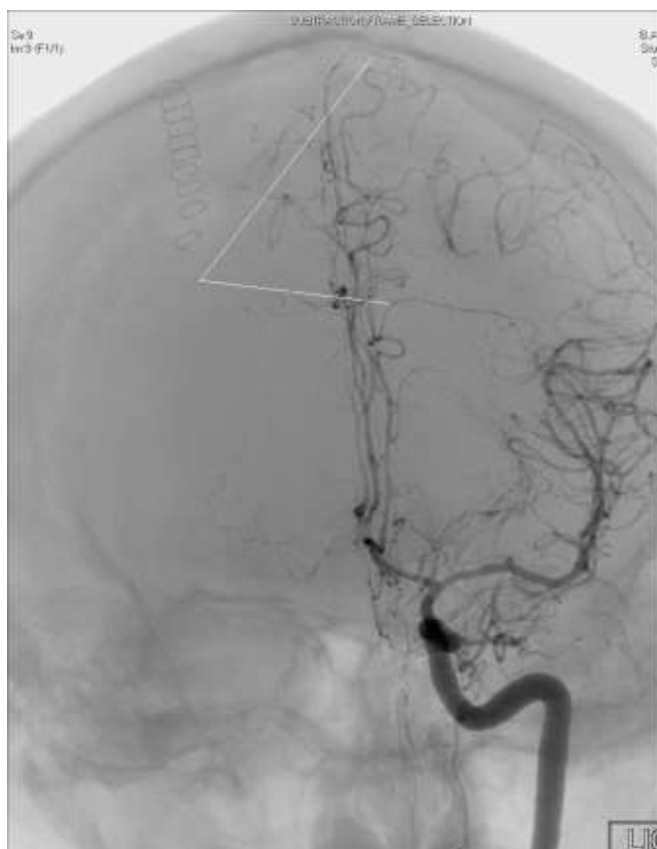


Figure 6. Primary CNS vasculitis. Cerebral angiography shows widespread vessel irregularity and beading.

PRIMARY LARGE VESSEL VASCULITIS

Giant cell arteritis (GCA) and Takayasu arteritis (TAK) are prototypical large vessel granulomatous vasculitides (LVV) that involve the aorta and its major branches however any size artery can be affected [1]. Imaging studies and fluorescein angiography studies have shown that ocular involvement in GCA can affect not only ophthalmic, but also medium caliber retinal arteries and multiple ciliary arteries and their smaller branches; blindness may result not only from LVV but injury to smaller ophthalmic branches [1]. Whereas GCA has a predilection for branches of the carotid and vertebral arteries and age at onset older than 50 years, often associated polymyalgia rheumatica, TAK is typified by involvement of proximal subclavian and carotid arteries and onset by age 40 years or less. Neuroimaging studies employing ultrasonography, high-resolution MRI, and ¹⁸fluorodeoxyglucose-positron emission tomography (FDG-PET) imaging are useful modalities to depict superficial cranial and extracranial vessels, and large vessel subclavian and aortic involvement in GCA and TAK [11].

CNS involvement in GCA, which results from thrombosis of the carotid and vertebral arteries rather intracranial arteritis, affects vessels that contain elastin, more specifically the internal elastic lamina that is absent from intracranial vessels more than 5 millimeters beyond the point of dural perforation. Reich and colleagues [12] and Gonzalez-Gay and co-workers [13] summarized the literature pertaining to stroke and GCA. Reports of CNS involvement were erroneously attributed to intracranial GCA rather than PCNSV [14-16]. Hellenhorset and colleagues [17] noted CNS events including stroke in 7.4% of 175 patients with confirmed GCA, as well as, and one patient with massive cerebral hemorrhage, two with stroke, and six with occlusive disease of the aortic arch. Caselli and colleagues [18] noted transient ischemic attacks or stroke in 7% of 166 patients with biopsy-proven GCA, among whom four had events in the vertebrobasilar system, and eight affected the carotid arterial system. With approximately 30% of patients manifesting neurological findings, the commonest of which were neuropathies of the arms and legs according to Caselli and colleagues [18], Salvarani and colleagues [19] observed that transient ischemic attacks or stroke in the territory of the carotid or vertebrobasilar arteries were less common neurological findings.

Gonzalez-Gay and co-workers [13] ascertained the frequency of cerebrovascular accidents (CVA) in association with GCA among 239 patients in a multicenter retrospective analysis to assess the features, therapeutic response, and predictors of visual loss and CVA. Eight patients (3%) developed CVA, equally divided between the vertebrobasilar and carotid territories. The ischemic events were preceded by other symptoms of arteritis by a median of 1.5 months, and were more frequent in those with visual involvement, especially permanent visual loss. Two patients with vertebrobasilar stroke died within one month despite aggressive corticosteroid therapy. Stepwise logistical regression analysis revealed visual loss and jaw claudication as the predictors of CVA.

Kerr and colleagues [20] summarized the clinical, laboratory and treatment responses of 60 patients with Takayasu arteritis (TAK) based upon the presence of symptoms and signs of ischemic, inflammatory large-vessel disease as well as supportive arteriographic findings, so noting 10 (17%) patients with either transient ischemic attacks or CVA, and carotid or vertebral artery disease, eight of whom corresponded to the neurological deficit. One of the

remaining two had hypertension, whereas the other patient had isolated stenosis of the innominate artery. Riehl [21], and Riehl and Brown [22] reported the clinical and pathological features of six patients with TAK noting widespread arteritis that not only involved the aortic arch and its tributaries by clinical and angiographic studies, but many other medium-, and large-sizes vessels. One patient with TAK manifested clinical and histopathological evidence of CNS involvement. That patient, a 43-year-old woman who presented with rapidly disappearing blood pressure and complained of spells of progressively worsening dizziness and dimness of vision, died of severe congestive heart failure. At postmortem examination, there was severe stenosis of the distal aorta, thrombosis of an aortic arch graft with nearly complete obstruction of all the great vessels by granulomatous panarteritis, as well as vasculitic involvement of the proximal portion of the left MCA, both PCA arteries, and anterior third of the basilar artery. There was massive infarction of the left temporoparietal region, the left half of the midbrain, brain stem, and cerebellum.

PRIMARY MEDIUM VESSEL VASCULITIS

Polyarteritis Nodosa (PAN) and Kawasaki disease (KD) are prototypical medium vessel vasculitides (MVV). Although any size of artery may be affected in this category, PAN is associated with necrotizing arteritis of medium or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules; however, it is not associated with anti-neutrophilic cytoplasmic antibodies (ANCA) [1]. By contrast, KD is an infantile or childhood disorder that is associated with the mucocutaneous lymph node syndrome, predominantly affecting medium and small arteries, occasionally coronary, the aorta, and other large arteries [1].

Among early postmortem series of PAN, Middleton and McCarter [23] noted CNS involvement due to arteritic lesions in one (Patient 1) that consisted of an occasional small artery. Brain tissue was not examined in one patient (Patient 2) and was not mentioned in another (Patient 3). Kernohan and Woltman [24] summarized the clinicopathological aspects of adult PAN in six patients studied at postmortem examination, estimating CNS involvement associated with stroke in about 8% of cases, and a combination of acute and chronic lesions that correlated with known exacerbations. In three patients, the peripheral nervous system (PNS) was widely involved at onset and at postmortem examination in addition to systemic involvement typically sparing the CNS, while in one patient, the PNS and CNS was involved alone. That patient was a 32-year-old man with onset of left arm and leg hemisensory loss and headache that progressed to paralysis. At postmortem examination there was marked thickening and near complete obliteration of the right MCA, less so on the left side, and obstruction of the right PCA by a recent thrombus, with a normal PCA on the left. Microscopic analysis showed subacute and chronic lesions of PAN.

According to Guillevin and co-workers [25], CNS involvement is rare but motor deficiencies, stroke and brain hemorrhages can occur as well as cognitive disturbances associated with abrupt memory loss and scattered T₂-weighted hyperintensities on brain MRI suggestive of cerebral vasculitis. CNS involvement including stroke was noted in 9.6% of 115 patients in the FVSG [26] with hepatitis B virus (HBV)-PAN between 1972 and 2002. The FVSG treatment protocol for HBV-PAN generally included cyclophosphamide,

corticosteroids and plasma exchange (PE), alone or together, followed by vidarabine, interferon-alpha (INF- α), or lamivudine, depending upon the time of diagnosis and different treatment protocols available [27-29]. So treated, nine patients (9.7%) who achieved remission subsequently relapsed, one of whom died of stroke, but the relationship between PAN and stroke could not be established. A retrospective study of 348 adult patients registered in the FVSG [30] who satisfied criteria for the diagnosis of PAN between 1963 and 2005 noted CNS involvement including stroke in 4.6% of patients overall, with a relatively equivalent frequency among patients with and without HBV-related illness.

PRIMARY SMALL VESSEL VASCULITIS

Small vessel vasculitis affects intraparenchymal arteries, arterioles, capillaries, and venules however medium-sized vessels and veins may also be involved [1]. Two major categories of SVV include ANCA-associated vasculitis (AAV) characterized by necrotizing vasculitis with few or no immune complex (IC) deposits predominantly affecting small arteries, arterioles, capillaries, and venules associated with myeloperoxidase (MPO) ANCA or proteinase 3 (PR3). IC vasculitis is characterized instead by marked vessel wall deposits of immunoglobulin (Ig) and complement. Granulomatosis with polyangiitis (GPA) (Wegener granulomatosis [WG] type), eosinophilic granulomatosis with polyangiitis (EGPA) [Churg-Strauss syndrome [CSS]], and microscopic polyangiitis (MPA) (microscopic polyarteritis) are prototypical AAV, while cryoglobulinemic vasculitis (CV), IgA vasculitis/Henoch-Schönlein purpura (IgAV/HSP), and hypocomplementemia urticarial vasculitis (HUV) associated with C1q antibodies comprise the IC vasculitides; HSP/IgAV is predominantly a disorder of childhood.

ANCA-ASSOCIATED VASCULITIS

Granulomatosis with Polyangiitis

GPA is characterized by necrotizing granulomatous inflammation of the upper and lower respiratory tracts and necrotizing vasculitis of small to medium arteries, arterioles, capillaries, veins, and venules together with glomerulonephritis [1]. Drachman [31] described a patient with one month of headache that awakened him from sleep followed by rhinitis, nasal obstruction, epistaxis, mononeuropathy multiplex, confusion and hypertension. Active arteritis and necrotizing granulomata were found in the brain but not in peripheral nerves. Based upon the observations in a single postmortem studied patient, cerebral involvement according to Drachman [31], depended upon a combination of four separate entities, 1) Frank vasculitis of larger arterial branches particularly over the surface of the brain as demonstrated in his patient report; 2) Ischemia in portions of the territory supplied by the affected vessels; 3) Subarachnoid hemorrhage and hypertensive encephalopathy evidenced by microscopic infarcts in close relation to arteries with fibrinoid impregnation of their walls; and 4) Meningeal infiltration by mononuclear cells. Nishino and colleagues [32] described neurological involvement in 34% of 324 consecutive patients with GPA at the Mayo Clinic

between 1973 and 1991 classified according to the American College of Rheumatology (ACR) [33] that included peripheral neuropathy in 16% compared to cerebrovascular events in 4% at some time in the course of illness, however rarely if ever at presentation. Of five patients with presumed clinical vasculitis, cerebral angiography was negative in two. Of twelve patients studied at postmortem examination, findings of vasculitis were detected in only two patients. Fauci and colleagues [34] noted nervous system involvement in 22% of 85 patients, 10 patients (12%) of whom had CNS involvement including strokes at some point in the illness although not specifically mentioned at presentation. Moore and co-workers [35] estimated the frequency of neurological abnormalities of GPA at 23% to 50%, citing possible mechanisms of neural dysfunction due to inflammation from primary sites, remote granuloma formation, and vasculitis. Drachman [31] opined that contiguous extension of necrotizing granulomas might account for 24% of all neurological complications including those of the CNS.

Extensive cerebral infarction in the territory of the bilateral ACA was ascertained in a 67-year-old man with frontal headache that preceded detection of a large midline frontal mass lesion on computed tomography (CT), and known biopsy-proven GPA and granulomatous vasculitis of the nasal cavity, liver and kidney. Postmortem examination showed cerebral infarction in the areas supplied by A2 branches of both ACA, with occlusion of large sized ACA vessels by organized mural thrombi, and fibrinoid necrosis in the arterial branches. There was similar vasculitis with multinucleated giant cells involving small arteries and veins diffusely in the frontal region of the brain suggesting contiguous spread from granulomatous nasal cavity lesions. Hoffman and colleagues [36] retrospectively assessed 180 patients with GPA for 6 months to 24 years noting nervous system involvement in 23% of patients, including CNS involvement and stroke in 8% of patients. A prospective analysis of clinical, electrophysiologic, radiologic, and serologic data of 128 GPA patients over 19 months [37] noted CNS involvement in 7%, the latter including granulomatous infiltration of frontobasal cortex arising from adjacent paranasal sinus granulomas, cerebral vasculitis, stroke, vascular myelopathy, and meningeal granulomatosis.

Microscopic Polyangiitis

Microscopic polyangiitis is a necrotizing AAV vasculitis with few or no IC deposits and commonly associated glomerulonephritis without granulomatous inflammation [1] Neither Davson and colleagues [38] who separated fourteen postmortem studied patients from 1934 to 1947 with MPA into two groups based upon the presence of severe widespread glomerular damage, nor Wainwright and Davson [39] who described MPA among 6 post-mortem studied patients from 1947 to 1948, mentioned CNS involvement, stroke onset or histopathologic brain involvement. Nor was there mention of patients with CNS involvement by Adu and co-workers [40] among 43 patients with renal histologic evidence of MPA. Savage and co-workers [41], who studied 34 patients with MPA, all of whom presented with clinical evidence of a systemic SVV predominantly affecting the skin and musculoskeletal system associated with focal necrotizing glomerulonephritis, cited CNS involvement at presentation in 18% of patients without specific mention of stroke. Serra and colleagues [42], who reported the presentation, histopathology and long-term outcome of 53 patients with MPO from 1965 to 1981, cited CNS involvement at presentation in 15% of patients, the findings of

which included stroke, convulsions, headache, confusion and drowsiness. Guillevin and co-workers [43] who reported the clinical and laboratory findings in 85 patients from the FVSG with MPA from 1969 to 1995, noted CNS involvement in 11.8% of patients at presentation and in 3% of 29 patients who experienced a relapse; however, there was no specific mention of stroke. Gayraud and co-workers [44] analyzed four prospective trials that included 278 patients with PAN, MPA and EGPA from 1980 to 1993, that reported stroke occurrence that accounted for 85 deaths. Villiger and Guillevin [45], who reviewed several retrospective European patient cohorts [40-43, 46], cited CNS involvement that included subarachnoid hemorrhage, cerebrovascular disease, meningitis and diffuse brain injury. Ben Sassi and colleagues [47] described intracerebral hemorrhage secondary to necrotizing vasculitis that similarly involved the nerves and muscles, citing three additional patients with hemorrhagic stroke due to MPA [48-50]. Ahn and colleagues [51] studied 55 patients with MPA, 69% of whom demonstrated perinuclear ANCA by immunofluorescence (IF) or MPO ANCA-seropositivity, noting CNS involvement in 5 (9.1%), none of whom developed end-stage renal disease. and one of whom died at follow-up without known autopsy.

Eosinophilic Granulomatosis with Polyangiitis

EGPA is a necrotizing vasculitis involving small to medium vessels that differs from GPA by the presence of eosinophil-rich necrotizing granulomatous inflammation of the respiratory tract in association with asthma and eosinophilia, and ANCA seropositivity when glomerulonephritis is present [1]. Churg and Strauss [52] described the clinical and postmortem findings of thirteen patients with asthma, fever, and hypereosinophilia. This was accompanied by eosinophilic exudation, fibrinoid change, and granulomatous proliferation that constituted the so called allergic granuloma. The latter were found within vessels walls and extravascular connective tissue of major organ systems. CNS organ manifestations were described in 8 (61.5%) of patients, and the cause of death in 3 patients who sustained cerebral hemorrhage or subarachnoid hemorrhage. Chumbley and co-workers [53] described 30 asthmatic patients from the Mayo Clinic over the period from 1950 to 1974 with necrotizing vasculitis of small arteries and veins, extravascular granulomas and infiltration of vessels and perivascular tissue eosinophilia. Lanham and colleagues [54], who emphasized that the combination of necrotizing vasculitis, tissue infiltration by eosinophils and extravascular granulomas suggested by Churg and Strauss [52] occurred contemporaneously in only a minority of patients, and that such histological findings could be encountered in other granulomatous, vasculitic and eosinophilic disorders in the absence of clinical asthma, allergic rhinitis, sinusitis, pulmonary infiltrates, and cardiac involvement pathognomonic of EGPA, noted CNS involvement in 25% of cases. Cerebral hemorrhage and infarction as a consequence of vasculitis or hypertension was a major cause of morbidity and mortality, accounting for 16% of deaths.

Sehgal and colleagues [55] noted neurological involvement among 14 patients with the clinical diagnosis of EGPA, three of whom had cerebral infarction two to fifteen years after initial diagnosis. The first patient was a 68-year-old woman with a stroke involving the territory of the MCA manifested as hemiparesis and aphasia. The second patient was a 34-year-old man with a right parietal lobe infarction pursuant to embolization of a left ventricular thrombus, manifested incoordination and hemisensory loss of the arm. The third patient was a

62-year-old woman with a thalamic infarction who manifested hemibody sensory loss. Guillevin and co-workers [56] studied 96 patients with EGPA between 1963 and 1995, noting ischemic stroke onset in 6 (6%) by brain computed tomography (CT). Mouthon and colleagues [57], reported 38 patients with EGPA from 1978 to 1998, citing CNS involvement in 3 elderly patients (7.9%) in whom CNS vasculitis was suggested by MRI of the brain in only one patient. Among 383 patients with EGPA enrolled in the FVSG Cohort [58] employing the definition of the ACR [59], CNS involvement was noted in 20 (5.2%) of patients at onset, with two-fold greater frequency of ANCA-seropositive serology than those ANCA-seronegative, but no specific mention of stroke frequency.

IMMUNE COMPLEX VASCULITIS

Cryoglobulinemic Vasculitis

Cryoglobulinemic vasculitis (CV) or CryoVas is typified by cryoglobulinemic IC deposited along small vessels predominantly arterioles, capillaries, veins, and venules in association with serum cryoglobulins. In mixed cryoglobulinemia (MC), the immune complex is composed of IgM and polyclonal IgG with rheumatoid factor activity often in association with hepatitis C virus (HCV) infection. Disease manifestations of cryoglobulinemia was noted in 206 of 443 (47%) patients [60] that included palpable purpura, 12 (6%) of whom had CNS involvement; HCV Infection was the main etiologic factor noted overall in 75% of the 443 patients. A concomitant autoimmune disorder was noted in 24%, hematologic disease in 7%, and essential cryoglobulinemia in 11% of the patients overall. The original patient described by Lerner [61], a 56-year-old man who presented with chest pain, purpuric rash and a cold precipitating serum protein, did not have symptoms or postmortem findings referable to the nervous system. Marshall [62] noted widespread cerebral purpura with hemorrhage ascribed to occlusion of small blood vessels by eosinophilic protein precipitates that correlated with the clinical presentation of progressively fatal coma.

Abramsky and Slavin [63] described three patients with MC and CNS involvement. The first was a 55-year-old woman with anarthria, hyperreflexia, bilateral Babinski signs and progressively fatal coma, who was later found at postmortem to have multiple thrombotic occlusions of small intracerebral blood vessels with adjacent foci of ischemia and marked demyelination. The second patient was a 50-year-old woman, who presented with a Wallenberg syndrome and was found to have MC with occlusion of the left posterior inferior artery at angiography. The third patient, a 50-year-old woman who presented with right hemiparesis and pyramidal signs, was found to have a cryoprecipitate and occlusion of the left MCA.

Gorevic and colleagues [64] summarized the clinical aspects of long-term followup of 40 patients with MC noting incidental CNS involvement at postmortem examination and a fatal stroke in another patient. Petty and co-workers [65] described a 35-year-old woman with type II cryoglobulinemia, headache, purpura, and seizures who was found to have multiple areas of T₂ and proton density signal abnormalities in the cerebral and cerebellar hemispheres, with a gyral pattern of enhancement in the cerebral cortex, a nodular pattern of enhancement in the

cerebellum, and early cortical infarction on right frontal brain biopsy without vasculitis; cerebral angiography was normal. HCV RNA was detected by polymerase chain reaction (PCR). Ince and colleagues [66] described a patient with paranoid psychosis and MC who was found to have right basal ganglia hemorrhage and multifocal small vessel occlusions by amorphous bland protein plugs and abundant Russell bodies present in arterioles and venules with relative sparing of capillaries.

Stroke was the presenting feature of CNS involvement in MC among two patients with lacunar infarcts and associated subcortical white matter changes on brain MRI [67, 68], and in one patient with a temporal arteritis-like syndrome with associated ischemic cerebral infarction [69]. Cacoub and co-workers [70] compared the features of patients with HCV examined for systemic vasculitis manifestations of PAN or MC noting two patients with cerebral vasculitis in the former versus none in the latter. Cacoub and colleagues [68] described CNS involvement in three patients with HCV infection and MC, one of whom was found to have a hemorrhagic lesion of the external capsule in association with pontine T₂ hyperintensities and parietal lobe infarcts. A second patient had a hemorrhagic occipital lobe lesion in association with cerebellar infarction and abnormal periventricular hyperintensities. A third patient had abnormal white matter hyperintensities.

Ferri and colleagues [71] studied the demographic, clinical, serologic features and survival of 231 patients with MC seen between 1972 and 2001, noting widespread vasculitis involving small- to medium-sized arteries, capillaries and venules with two or more visceral organs including the kidney, gut, and lung, noting no CNS manifestations of MC with exception of focal dystonia during interferon treatment in one patient [71]. Filipini and colleagues [72] reported a 63-year-old woman with acute severe encephalopathy HCV-related MC, dysarthria and hemiplegia. Frago and co-workers [73] reported a 35-year-old woman with dysarthria, left facial and left limb hemiparesis and hemisensory loss with MC in whom MRI showed ischemic lesions in the tail of the right caudate nucleus, corona radiata and posteromedial putamen. Casato and co-workers [74] reported the CNS findings in 40 patients with HCV-related CV in a multicenter, case-control study using MRI and neuropsychological testing. Although none evidenced cerebral infarction, small white matter lesions were found in all HCV-related MC subjects, a higher mean number of white matter intensities compared to HCV and healthy controls.

Terrier and co-workers [75] analyzed the data of 242 patients registered in the CryoVas survey ascertaining CNS involvement in 5 (2%) patients without specifying whether stroke was encountered. Terrier and Cacoub [76] reviewed CNS findings in HCV-seronegative and seropositive MC in CryoVas noting 2 (0.7%) patients among the former with CNS involvement, compared to 9 (5%) patients in the latter group evidencing CNS involvement but without further mention as to presence of stroke in any of the patients.

Hypocomplementemic Urticarial Vasculitis/C1q

Hypocomplementemic urticarial vasculitis/C1q presents with recurrent attacks of erythematous, urticarial and hemorrhagic skin lesions lasting up to 24 hours at a time, associated with recurrent attacks of fever, joint swelling, and variable abdominal distress. Circulating hypocomplementemia and C1q antibodies are associated with necrotizing SVV in skin biopsy tissue affecting arterioles, capillaries, and venules [1]. Apart from orbital

pseudotumor with ptosis, diplopia, and headache in one patient with HUV syndrome, there was no mention of CNS involvement or stroke in a review of 18 patients with HUV/C1q [77]. Nor was stroke mentioned in a review of four patients studied at postmortem examination with HUV/C1q [78]. Buck and colleagues [79] cited rare CNS manifestations in HUV/C1q including aseptic meningitis, pseudotumor cerebri, transverse myelitis but no mention of stroke or cerebral vasculitis. Grotz and colleagues [80] did not mention stroke or CNS vasculitis in a case report and review of the literature of HUV/C1q. In a review of HUV/C1q, Davies and Brewer [81] cited CNS involvement as “very rare”, attributing development of pseudotumor cerebri to vasculitis of the venous sinus system. Ludivico and colleagues [82] suggested that chronic pseudotumor cerebri in their patients was a diagnosis of exclusion and possibly related to chronic corticosteroid use, but did not cite patients with cerebral vasculitis or stroke.

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Chapter 78

STROKE AND PULMONARY EMBOLISM OUTCOMES OR COMPLICATIONS ASSOCIATED WITH ICD DEVICE IMPLANTATION

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ABSTRACT

Pacemaker and implantable cardioverter defibrillator device use has increased exponentially since their introduction. The devices and their placement process, in large part intravenous, make them susceptible as a nidus for thrombus formation and subsequent embolization to the pulmonary system or to the brain through patency in the interatrial septum. Available data examining this association and the role played by other clinical risk factors are divided. Through this chapter, we provide a comprehensive review and understanding of available evidence about the embolic risk associated with implantation of such devices and associated risk factors, mainly the PFO. We also discuss the various treatment options, both medical and interventional, with respect to risk of stroke or pulmonary embolism.

Keywords: pacemaker, implantable cardioverter defibrillator, stroke, transient ischemic attack, patent foramen ovale, atrial fibrillation

INTRODUCTION

During the early period of transvenous catheter implantation, unexpectedly few cases of embolism were reported. The ‘look out’ for adverse events in patients with implanted intravenous lead mainly centered around the complications associated with the mechanical

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properties, such as electrode displacement, myocardial perforation, electrode fracture, pulse generator extrusion, infection, generator failure, electrode induced arrhythmia, etc. [1-4] The absence of literature about possible thrombus/embolic complications associated with device implantation attracted an editorial By Dr. Kurt Amplatz [5] in response to an article titled 'Clotting on the outer surfaces of vascular catheters,' an experiment on dogs showing a possible thrombogenic property of catheter implantation in vivo.[6] Two months later in 1968, the first case of embolization followed by pulmonary infarction due to thrombus formation around the catheter in a human was reported by Prozan GB et al.[7] In the absence of any other predisposing factor for pulmonary thromboembolism (PTE) in that patient, the needle of suspicion went to frequent sepsis associated with the catheter material as earlier observed from the use of catheter (of similar material) for a ventriculo auriculostomy procedure in children.[8, 9] This was followed by another case, where the possibility of emboli from the right side of the heart was suspected, instead of only the peripheral veins, as envisaged until that time.[10] Following these two cases, an association between device implantation, resulting in a foreign body being left *in situ* predisposing the mechanism responsible for embolic risk, and stroke/pulmonary embolism (PE) resulting in life threatening complication was visualized.

Unlike PE, the stroke associated with right heart occurs via atrial septal defect (ASD), mainly the most common defect, patent foramen ovale (PFO). This anatomic entity was first described in 1564, and its role as conduit for passage of emboli was brought to attention in 1877 in an autopsy study. Any relation of right heart device associated with stroke is viewed in conjunction with the presence of PFO. The role of PFO in stroke, especially the cryptogenic stroke, has been matter of long debate. More so with the advent of PFO closure devices which promised to reduce the stroke risk in patients with PFO. Hence, the discussion about stroke associated with device implantation may be conveniently divided into two major parts. First, the role of the pacemaker or implantable cardioverter defibrillator (ICD) device, its components or its placement as the source of embolus, and second, the PFO is believed to act as a conduit for passage of emboli through the shunt resulting in adverse neurological events.

DEVICE AND THROMBOGENIC RISK

A pacemaker or ICD device is required to provide required electrical stimulation in the form of cardioversion, defibrillation or pacing of the heart. For the sake of simplicity, we include all such devices which require a lead/extension in the right heart. These include ICD, automated implantable cardioverter defibrillator (AICD), pacemakers and others.

The insulation material covering the leads is the part of the device which can directly cause thrombogenic activity and act as a foreign body directly in contact with blood. Lagergren H et al. in mid 1960s, described the phenomenon of deposition of fibrin over the leads within 12 hours of implantation.[11] The incorporation of the lead within venous tissue was found to be prominent at the curves at the superior vena cava and entrance in the right atrium. At this time, polyethylene material was used as insulating material which was gradually replaced by silicone and polyurethane (introduced in 1979).[12] The later

transformation to other insulating materials occurred because of better bio stability and mechanical properties associated with the new material, rather than due to the risk of thrombus formation and embolism. Presently, a combination of polyurethane, silicone and fluoropolymer materials is used to manufacture the lead insulation. The change in lead materials answered many questions about bio-mechanical issues associated with device implantation, but evidence regarding the changes to the thrombogenic potential has been lacking. While the thromboresistance was reported to be comparable between silicone and polyurethane leads in early study [13], a comparative thrombogenicity study later done on pigs by Palatianos GM et al. found polyurethane to be more thromboresistant as compared to silicone.[14] Nevertheless, there was no evidence showing the new materials in anyway behaved worse than the older polyethylene material, and not surprisingly, newer materials got wider acceptance and gradually replaced the older insulation material.

Theoretically, the procedure of insertion of a cardiac lead does seem to fulfill the three criteria of Virchow's triad, i.e., injury to vessel wall, possible flow stasis and hypercoagulability, which predispose the patient to a thrombotic event. While the first two components are part of the procedure and its consequence, the third (hypercoagulability) criterion has been documented through studies.[15, 16] Even though symptoms of pulmonary embolism can be seen in only 0.6% to 3.5% of patients, the incidence of asymptomatic thromboembolism can be as high as 48%, often in older patients and those with congestive heart failure, ischemic and valvular disease.[17-20] In earlier studies, venous thromboembolism (VTE) was reported after insertion of pacemaker lead/wires.[12, 20-26] But those earlier studies were either case reports or studies with a limited number of patients and lacking multivariate analysis. Importantly, even though these studies point towards development of prothrombotic events in such patients, it has not been shown to result in thrombosis and thromboembolism.

As for the thrombogenic risk of intravenous leads, there is broad consensus on a few basic points. Platelet deposition and intravascular coagulation occur early after pacemaker implantation and symptomatic embolism is a rare occurrence in patients undergoing implantation, though subclinical/asymptomatic findings or venous obstruction/thrombosis can be observed with varying frequency during autopsy or with echocardiography.[27-30] Harcombe et al. studied 2,621 patients from 1984 through 1994 and followed them for at least one year to study various complications. They reported a 'miscellaneous' complication rate of only 0.3% (7 of 2,621) with no specific embolic complications.[31] But the study was designed to mainly look for complications such as infection and other lead structure associated complications. Lead infection can be an additional source of emboli formation in patients with intravenous cardiac lead implantation, with as many as 27% of patients reported to have pulmonary embolism and 14% reported to have systemic embolization following lead infection.[32] A recent echocardiographic study on 1,084 patients with device implantation found that out of 32 patients with visualized thrombus on leads, 17 patients had endocarditis.[33]

More recently, a multicenter study by Khairy et al on 202 patients showed that congenital cardiac defect patients who had a transvenous lead had more than 2-fold increase in the risk of thromboembolic event, as compared to patients with an endocardial or no lead.[34] In their study, the higher probability of an embolic event was seen even after adjusting for baseline clinical risk factors, but the significance was lost after adjusting further for important stroke

related factors such as past thromboembolic event, Atrial Fibrillation and other variables. Conversely, a study by De Simone et al. of 6,075 patients with device implantation yielded similar results with PFO patients having a higher rate of stroke/TIA as a future event compared to patients without PFO, even after adjusting for other risk factors (8.2% vs 2.0%; HR 3.36; 95% CI 2.23-5.07; p-values < 0.0001). [35] This study raised important questions about the risk of embolism associated with the device in the presence of PFO. However, in a subsequent study done by the same group with the same cohort, they opined that the increase in risk of stroke/TIA may have an association with PFO itself rather than the device. [36] They studied various characteristics of the device and its possible association with neurological events and found that the tined lead, which anyway is losing prominence and is gradually being replaced by fixated leads, was an independent predictor for stroke in patients with a device. Furthermore, in a similar study including the same cohort, no difference in PE was detected between the patients with device (or device related characteristics) and the age/gender matched general population. Also, it was observed that most of the patients (84%) with PE had other established reasons for the embolic event. [37] Moreover, even the abandoned leads were not found to be associated with thromboembolic complications when patients with abandoned leads were followed for a period of 3 years. [38] Overall, no positive independent association between device and embolic event (stroke or PE) has been confirmed through recent large studies, although none of these studies specifically examined the possibility of a device or its components (and not PFO) as the cause of prospective neurological events.

One recent propensity matched study of 2,921 patients (device versus no device group) specifically scrutinized the possibility of the device as the source of stroke/TIA in PFO patients and found no difference in stroke, TIA or both outcomes between patients.[39] They included medication history, excluded patients with prior neurological events, and accounted for patients who had PFO closure after inclusion in the study. The major limitation was the low event rate which may preclude any significant result between the two groups, but the propensity matching made it as close to a randomized trial as possible with a retrospective study. Nevertheless, this was the largest recent study designed to specifically look into devices as the possible cause for embolism in the patient population.

Table. 1.

Study, Year	Patient Cohort, Number	Exclusion Criteria	Age (years), Male (%)	Relevant Characteristics	Follow-up	Outcomes
Khairy, P. et al, 2006 [34]	Patients with any Intracardiac shunt grouped into transvenous, epicardial and no leads, 202 patients	Patent ductus arteriosus and PFO	26.19±14.06, 96 (47.52)	Documented Right-to-left shunt - 149 (73.53%), Atrial Fibrillation / Flutter – 63 (31.19%)	12.66±15.0 8 years	After multivariate adjustment - point estimate for transvenous lead as independent risk factor for systemic thromboembolic episode (HR: 2.1;95% CI: 0.6 to 7.1; P=0.2274)

Study, Year	Patient Cohort, Number	Exclusion Criteria	Age (years), Male (%)	Relevant Characteristics	Follow-up	Outcomes
Glickson, M. et al., 2009 [38]	78 ICD patients with 101 abandoned leads	-	63±14, 63 (81)	-	3.1±2.0 years	No clinically apparent thromboembolic complications due to abandoned leads
DeSimone, C.V. et al., 2013 [35]	All patients with transvenous implantation of pacemaker / ICD, 6075 patients	Patients lacking details about timing, mechanism, or cause of stroke/TIA (40 patients), unconfirmed PFO (74 patients)	66.87±16.90, 3891 (64.05)	Atrial Fibrillation - 2672 (43.98%), Prior Stroke /TIA - 910 (15%),	4.7±3.1 years	After multivariate adjustment - association between PFO and stroke/TIA in patients with endocardial leads (8.2% vs. 2.0%: HR: 3.30; 95% CI: 2.19–4.96; $P<0.0001$)
Vaidya, V.R. et al., 2014 [36]	All patients with transvenous implantation of pacemaker/ ICD, 5646 patients	Patients lacking details about timing, mechanism, or cause of stroke/TIA	67.3±16.3, 3613 (64)	Atrial Fibrillation - 2535 (45%), Prior Stroke /TIA - 852 (15%)	-	After multivariate analysis - older age, female sex, documented PFO, prior history of stroke/TIA presence of timed leads (HR: 1.41; 95% CI: 1.00–1.97; $p=0.049$) were associated with an increased rate of stroke/TIA
Poddar, K.L. et al., 2014 [39]	All patients with PFO divided into device and no device groups (231 patients in each group, 2921 patients)	Prior Neurological event, PFO closure during follow up (if no event before closure)	76.33±13.75, 271 (58.66)	Atrial Fibrillation - 264 (57.14%)	At least 4 years follow up completed for each patient	Propensity matched analysis - Stroke/TIA outcomes were not different between the two groups (4.3% vs. 5.2%; $P=0.83$)
Noheria, A. et al., 2015 [37]	All patients with transvenous implantation of CIED, 5646 patients	-	67.3±16.3, 3614 (64)	Atrial Fibrillation - 2566 (45%), Prior Stroke/TIA - 849 (15%)	Mean follow up of 4.69 years	No statistical association between PE event and number of leads or type of device
PFO: Patent foramen ovale; ICD; Implantable cardioverter defibrillator; TIA: Transient ischemic event; CIED; Cardiac implantable electronic devices; PE: Pulmonary embolism						

Considering the absence of consensus on definite embolic risk factors and the fact that these studies leaves us with little evidence to independently link a device or its components

with stroke, it can be safely concluded that the risk of thromboembolism in patients and who require a pacemaker or ICD are most likely multifactorial.[40, 41] Therefore we divert our focus to other pertinent and accepted risk factors associated with a subsequent neurological event.

DEVICE AND PATENT FORAMEN OVALE

PFO, a defect due to incomplete fusion of septum primum and septum secundum, is a common benign entity present in around 20-25% of the population and is mostly an incidental finding during echocardiography as part of clinical investigations or during autopsy. The presence of the channel, which allows for paradoxical embolism, generated interest to evaluate the relationship between PFO and stroke. Brogno et al. showed evidence of the passage of thrombus through the PFO [42, 43], even though the probable association of PFO with CS was first reported much earlier in 1988 by Lechat et al.[77] It was later studied by others, where PFO as an independent risk factor of stroke was discussed, but with conflicting evidence.[44-46] Overall, various studies associated PFO with thromboembolism, with many of those showing an association between PFO and CS, suggesting a role for PFO in stroke pathophysiology.[44] A meta-analysis of retrospective studies showed that patients <55 years who sustained a CS had a PFO prevalence 6 times greater than that of patients with other forms of stroke, while Handke et al. showed that the prevalence was higher among patients with CS than among those with stroke of known cause in both younger and older patients, suggesting paradoxical embolism as a cause of stroke [47-51]. These studies suggest that PFO is more common in patients with a CS (approximately 50% to 60% vs. 20% to 25%) or in patients with stroke of determined origin as compared to the general population. In contrast, a recent trial of 909 patients concluded that alternative etiologies, and not PFO, were mostly responsible for stroke/TIA in patients with documented PFO.[52] Furthermore, a recent meta-analysis of the relevant studies (recurrent cerebrovascular events and ischemic stroke or TIA in patients with PFO) from 1999 and beyond, found that patients with PFO and stroke do not have a higher propensity of either stroke or the combined end point of stroke/TIA as compared to patients with PFO (RR 1.18; 95% confidence interval (CI) 0.78–1.79; P 0.43).[53]

Conflicting results are mainly attributed to vastly different and weaknesses in methodologies, use of mainly retrospective and circumstantial evidence and poor control selection. Also, the studies were unadjusted for important co-morbidities and parameters, apart from limitations associated with low outcomes, an inherent limitation considering the event rate, decreasing the possibility of statistical significance. Overall, the relation has oscillated between causal, incidental or even no connection between the two entities in question. More so, considering that PFO is a normal anatomic variant and can exist in one-third of any study population by chance.[44] This makes the studies prone to under recognition of PFO in the comparison group apart from the possibility of selective referral.[54] The role of the investigation methodology may also be very crucial in determining the scope of risk.[53]

Transesophageal echocardiography (TEE) remains the mainstay investigation in patients with PFO as transthoracic echocardiography (TTE) is largely deficient for delineating PFO

and its characteristics when compared to TEE. TEE can be used to find PFO in different ways: finding abnormal flow through use of color doppler, direct B-mode visualization of the separation of the septum primum and septum secundum by omniplane transducer, and also by visualization of movement of injected saline. While TEE has a sensitivity of 89% and specificity of 100% in detecting PFOs compared with autopsy diagnosis, TCD can detect around 90-100% PFOs. TCD has been particularly helpful for visualizing small PFOs mainly because of the possibility of eliciting stronger Valsalva Maneuvers which can be further confirmed by the waveform. In one of the study, It was even advised that TCD be used as the first choice for screening patients with suspected PFO, as they found that in 4.8% of cases, TEE failed to reveal the contrast passage unlike TCD.[55] Even though TEE may sometimes lead to gross underrepresentation of presence of thrombus in patient with device implantation [56], it is still considered the gold standard for visualization in case of suspected emboli or stroke in such patients. The benefit offered by TEE is the additional information it provides apart from the advantage of direct visualization, i.e., atrial details and possible identification of atrial or aortic source of emboli such as ASA.

Various PFO specific parameters were studied to understand the anatomy associated with the stroke risk, but clearly, the 'defect' feature which has received maximum attention is the presence of concomitant atrial septal aneurysm (ASA) [57, 58] and to large extent, the shunt/PFO size, which may, in turn, be co-related to the amount of shunt across the defect.[59] Earlier studies have shown significant positive association of the shunt size with neurological event, or a trend towards it.[58, 60-65] The reason for discrepancies between the findings seems mainly due to the different definitions for the degree of shunting used in these studies. Goel S et al. studied consecutive 102 patients, without prior history of neurological event, and divided the shunts into mild (3 to 9 microbubbles in the left atrium at rest or after valsalva maneuver), moderate (10 to 30 microbubbles) and severe (30 or more microbubbles) categories and co-related it with the size of PFO and found that degree of shunting worsened with increasing PFO size.[59] More recently, in a meta-analysis done on 4251 patients, transcranial doppler ultrasound (TCD) studies suggested an increased risk of stroke/TIA in patients with moderate/severe shunt as compared to patients with small shunt (HR 2.91; 95% CI 1.32 – 6.41; P-Value 0.008).[53]

ASA, a congenital malformation of the atrial septum characterized by the bulging of the septum overlying the fossa ovalis into either atrial chamber, has also been implicated to be, possibly, the only potential cardioembolic source of thrombi (irrespective of presence of PFO) in patients with cerebral ischemia [66], as its thrombus can be observed in the base of aneurysm or as fibrin-thrombus tags on the convex surface of ASA.[67] Its association with PFO is not only shown to be increasingly found along with PFO [68], but also to synergistically increase the chance of embolism while co-occurring with PFO.[50]

Various prospective studies which dealt with the question of increased risk of stroke with both PFO and ASA were ambiguous. While some studies showed the combination as predictor of increased stroke [50, 69], other studies found the association to be insignificant.[69-71] The French PFO/atrial septal aneurysm (ASA) published in 1995 concluded that the combination of ASA and PFO is predictive of higher recurrent stroke risk [72], but the PFO in cryptogenic stroke (PICSS) study published in 2002 did not find any relation of PFO with stroke, with or without ASA.[69] Devuyst G et al. appreciated the difference in mean age between the patients of these two studies and performed a meta-

analysis using this two studies and concluded that it is possible that the association may be influenced by the age group of the patients. [73]

OTHER RISK FACTORS

Age has been found to be an important factor which seems to follow an inverse relation as far as stroke is concerned in PFO population. Meta-analysis by JR Overell et al. clearly shows how age differs across between the 'positive causal' relation against the 'neutral or negative studies'. [51] They divided more than 15 studies from the two mentioned groups and found mean age to be to be significantly different between the two groups, 44.8 years in 'positive causal' studies [57, 74-80] versus 61.1 years in 'neutral or negative' studies [81-87] thus indicating that the embolic event and PFO may be associated in young population, but less so in the older age group. In an analysis between cryptogenic and conventional stroke, more than 2 fold increase in the prevalence of PFO was observed in young population (< 55 years) as compared to older population (≥ 55 years of age). [44] This possibility can be explained by the fact that the prevalence of PFO decreases with the age. [88] Interestingly, the decrease of PFO prevalence with age, coupled with the finding of decreased association of PFO with stroke in the elderly, does substantiates that in the young, PFO may actually not be a silent bystander. But the same can be countered by the fact that as age advances, other thrombotic/embolic risk factors such as atrial fibrillation, atherosclerosis, diabetes and other co-morbidities may actually offset the role of PFO as a causal factor for any embolic event, and thus make PFO less relevant for any neurological event probability.

Apart from age, the other important concurrent pathology with respect to stroke associated with device implantation is arrhythmia, mainly AF, which can act as a precursor for an embolic event. [89] With the help of a gene expression profile study, it was found that nearly 58% of cryptogenic strokes actually have a undetected cardioembolic cause. [90] Many studies confirmed the presence of short lived arrhythmic properties, such as paroxysmal atrial fibrillation, in as much as 20% of cryptogenic stroke patients when patients underwent prolonged monitoring. [91-99] This is apart from findings of EMBRACE and CRYSTAL-AF trials which showed an increase in AF detection with prolonged monitoring. [100, 101] Additional arrhythmias such as paroxysmal atrial flutter [102], paroxysmal supraventricular tachycardia [103-105], sick sinus syndrome [106, 107] and atrial high-rate episodes in patients with cardiac pacemakers [108] also add to the unknown causes of cryptogenic stroke, which constitute a large proportion of stroke population ranging from 20% to as high as 40%. [109, 110] Hence, it is highly likely that cryptogenic stroke attributed or attributable to devices may actually have an undetected arrhythmic origin and thus the possibility of missed arrhythmia in such patients needs to be acknowledged.

Here it is important to note that the term cryptogenic stroke actually encompasses stroke of undetermined origin under various conditions, If the diagnostic assessment of the stroke event is incomplete, or after extensive investigation no cause could be found, or when a cause for stroke is not specified because more than one possible reason is found during investigation. [111] In a comprehensive review published in *LANCET Neurology*, a more specific term, (ESUS) was used to clarify the concept of cryptogenic stroke [112], as is understood in normal clinical practice. According to them, a diagnosis of ESUS should be made after thorough investigation has been done to exclude major risk cardioembolic sources,

occlusive atherosclerosis, and lacunar stroke. It may be beneficial to undertake similar investigation as designed to diagnose embolic stroke of undetermined source when planning a device implantation procedure in high risk patients, or those with prior history of embolic event.

Procoagulant conditions may also have the propensity to impact the management strategy of patients who may be more prone to thrombotic events such as device implantation. Though there are many such factors which can be classified as being prothrombotic, ranging from complex inherited/acquired thrombophilic conditions and antiphospholipid antibody syndrome to more common entities such as malignancy, smoking, OCP use and even female gender.

The association of a hypercoagulable state and stroke had been studied in detail, but studies looking specifically at the risk of clinical stroke/TIA in patients with a hypercoagulable state and subsequent device implantation were not done. Considering the low event outcome coupled with chances of few qualifying patients, such a study seems unlikely, and in its absence, focus may be given to studies dealing with common hypercoagulability status and stroke in PFO patients. Few independent retrospective studies analyzed different prothrombotic markers in patients with cryptogenic stroke and PFO: antiphospholipid antibodies (APS) [113-117], protein C and protein S deficiencies [116, 117], factor V Leiden mutation (FV_{G1691A}) [116, 118-121] and prothrombin gene mutation (PT_{G20210A}) [118, 119, 121], but the studies included either very few numbers of patients or the designs were not robust enough to be considered relevant to be incorporated into clinical practice.[122] Out of the mentioned conditions, the most common entities are heterozygosity for factor V Leiden (FVL) and PT, the two most common genetic risk factors, and also the antiphospholipid antibody syndrome (APS), one of the most important acquired risk factors for thrombosis.

FVL, present in as high as 5% in white Americans is notably the most common prothrombotic genetic predisposition.[123] Meta-analysis in the early 2000s have supported an association between FVL and first ischemic stroke [124-126] but two recent large studies were not able to find any association between FVL and first ischemic stroke and recurrent stroke. [127, 128] As far as PFO patients are concerned, the absence of association is further confirmed through various studies along with meta-analysis done by Pazzini et al.[129, 130] Overall, no conclusive proof was found to positively link FVL with risk of stroke in PFO patients.

Unlike FVL, meta-analyses showed a degree of association (OR 2.31; 95% CI 1.20 – 4.43) of prothrombin mutation with stroke risk in PFO patients, when PFO related stroke patients were compared with non-PFO stroke patients, although the largest study among them on 416 patients [119] apparently showed no association (OR 2.01; 95% CI 0.69 – 5.89). The most recent study published in 2012 by Favaretto et al. consisting of 340 patients, who also underwent TCD, did show association of G20210A mutation with cryptogenic stroke (OR: 2.97; 95% CI: 1.32-6.69) but no association with PFO (OR: 0.88; 95% CI: 0.39–2.01) or the RLS grading as assessed by TCD.[130]

Anti phospholipid syndrome, an acquired autoimmune disorder involving systemic circulation, by far seems to be strongly associated with chance of stroke in patients, with study findings it to be present in around 20% of young patients with thrombophilia.[131, 132] In a large systematic review of 5,217 patients from 43 different studies, the author found a 5-

fold increase in stroke/TIA with presence of aPL.[133] Furthermore, Pezzini et al. showed an association of neurological outcomes with the classical risk factors along with circulating antiphospholipid antibodies (aPL). They found that even after adjusting for other predictors, patients with persistent aPL has around 2 to 3 times higher risk of neurological events.[129] Overall, the literature seems to be consistent with a positive association of aPL with stroke [134-137], along with association between PFO and APS [138]. In contrast to that, Rajamani Kumar et al. provided results from a large study [139] which included patients who had TEE as part of the Patent Foramen Ovale in Cryptogenic Stroke Study (PICSS), and the aPL measurement through phospholipid and Antiphospholipid Antibodies and Stroke Study (APASS) (both studies relying on the Warfarin Aspirin Recurrent Stroke Study (WARSS) trial for patient recruitment). [69, 140, 141] In their analysis of 530 patients, they did not find an association of stroke with concomitant findings of aPL and PFO.

Overall, despite the association of hypercoagulable state with stroke risk in the general population, studies showing an additive or causal relationship of such prothrombotic states with device implantation and a neurological event have been limited to few cases. [142-144] Nevertheless, it is highly plausible that that insertion of an intravenous lead may tilt the balance towards higher risk in this hypercoagulable subset of patients. In lieu of absence of conclusive data, the insertion of an intravenous lead/device may be considered an additive risk factor in presence of a prothrombotic state, more so in the presence of past history of stroke or an associated clinical risk factor. Under such circumstances, the decision should be case specific. When in doubt, further investigation for prothrombotic status and markers should be done to help choose from various medical and interventional options and to form a comprehensive secondary preventive strategy.

MANAGEMENT

The current PACES/HRS Expert Consensus Statement on the Recognition and Management of Arrhythmias in Adult Congenital Heart Disease, endorsed by the governing bodies of PACES, HRS, the American College of Cardiology (ACC), the American Heart Association (AHA), the European Heart Rhythm Association (EHRA), the Canadian Heart Rhythm Society (CHRS), and the International Society for Adult Congenital Heart Disease (ISACHD), classify the placement of endocardial leads in the presence of CHD and intracardiac shunts under class III recommendation with level of evidence B.[145] The recommendation relied on advice from the earlier Canadian consensus statement of 2008 and ACC/AHA guidelines published in 2008.[146, 147] While the Canadian Consensus statement recommends avoiding transvenous pacing in patients with unrepaired ASD (level of evidence C), the ACC/AHA consensus statement advocated use of an epicardial pacemaker in patients with intracardiac shunt (Level of evidence B). This points to that fact that in the last few years either the evidence in favor of using device placement in patients with CHD are few or are conflicting in nature. Until further data are published or randomized trials are done, it is recommended to tread with caution, and it may be beneficial to thoroughly investigate each patient to look for pro-thrombotic/embolic risk factors and direct the management accordingly.

Medical management of patients undergoing device implantation largely depends upon their thromboembolic risk. In patients considered high thromboembolic risk and on oral anticoagulation (OAC) therapy, suspension of OAC treatment in lieu of 'bridging' heparin during the procedure was the norm, despite various studies finding no increased risk of bleeding with continued use of warfarin during the procedure.[148-152] Ghanbari Hamid et al. did a meta-analysis using available studies and concluded against use of heparin 'bridging' during the implantation procedure.[153] In 2013, a randomized trial confirmed the results as they found significant reduction in clinically significant hematoma in the warfarin group, as compared to the heparin 'bridge' group.[154] Pending further evidence, continuing with warfarin (anti-coagulant) seems to be the preferred option, even though individual thromboembolic risk assessment may provide additional information to direct intra- and post-procedural management.

The other important role of medical or interventional management deals with patients with suspected or confirmed PFO who are undergoing device implantation, as PFO is widely considered an additional risk factor in patients undergoing device implantation. Different medical management strategies have been studied with respect to this patient population with use anti-platelets or anti-coagulants. The two major studies (Patent Foramen Ovale in Cryptogenic Stroke Study (PICSS) and Spanish Multicenter Study (CODICIA)) concerning the use of medications in these patients found similar stroke recurrence rates among various patient groups. No difference was found in outcome rates between all patients taking aspirin or warfarin (2-year event rates 14.8% versus 15.4%; hazard ratio 0.96; P 0.84; 95% CI 0.62 to 1.48) and also in the cryptogenic stroke subset of patients (2-year event rates 14.3% versus 12.7%; hazard ratio 1.17; 95% CI 0.60 to 2.37; P 0.65). Similarly no difference in outcome was observed between patients with massive, non-massive and no right-to-left shunt (3.4% vs 2.3% vs 4.5%, respectively; p 0.75). Similar results were obtained in patients with PFO and ASA as compared to those with ASA alone and across younger and the overall population.[69, 155] Generally, it can be concluded that in most of the studies significant benefit of one medical treatment over the other does not exist.

At present guidelines suggest use of anti-platelets for patients with ischemic stroke in the absence of an identified embolic source.[156] Until the results of 'RE-ESPECT-ESUS' trials provide further evidence it may be beneficial to use anti-coagulants in patients who have PFO and identified cases of embolic strokes of undetermined sources along.[112, 157] As for AF, recent guidelines recommend use of CHA2DS2-VASc for risk stratification for the possibility of stroke occurrence.[158] Other risk scoring system, namely CHADS₂ score [159], the Anticoagulation and Risk Factors in Atrial Fibrillation (ATRIA) stroke score [160], the Qstroke score [161] are also studied, but CHA2DS2 risk score remains the most widely used and recommended score system to stratify AF patients for optimal therapy. Overall, the medical management in these patients should be best advised keeping in mind the associated clinical features such as thrombogenic potential of concomitant risk factors such as atrial fibrillation, other co-morbid conditions and risk of bleeding, and other adverse complications associated with the use of anti-coagulant or anti-platelet use.[162]

Device closure of PFO got overt attention in recent times with the introduction of closure devices, i.e., Amplatzer PFO occluder (St. Jude Medical), STARflex and others. It was presumed that device closure of PFO may be advantageous to groups of patients undergoing device implantation, as PFO is invariably present in a large number of patients undergoing implantation, and its closure may help to block the conduit of emboli through the defect.

Backed by the studies that showed probable benefits of closing the PFO, it was speculated that closure of a defect may be beneficial, especially in cases of cryptogenic stroke, with introduction of the closure devices. Though all three recent major trials comparing outcomes of closure devices with medical therapy [163-165] failed to show significant benefits with closure of defects, all of them were consistent with regards to patients in medical management groups experiencing a higher number of events. Also the events per patient year were consistently higher with medical therapy, but the quantitative (not statistical) benefit, to an extent, is being offset by the higher number of new onset atrial fibrillation cases.[166]

When the stratified analysis of the two trials using the Amplatzer PFO Occluder device was performed, the results favored the use of the device as compared to medical management, but only with respect to stroke (HR 0.44; 95% CI 0.20 – 0.9; P-value 0.04) and not TIA (HR 0.8; 95% CI 0.37 – 1.74; P-value 0.58). This result seems to be consistent with the pooled analysis of data from 11 observational studies by Capodanno D et al.[167] The RESPECT trial did show significantly better outcomes with respect to stroke in the ‘as-treated’ cohort (HR 0.27; 95% CI 0.10 – 0.75; P-value 0.007), which in turn seems to be influenced by better performances in the substantial shunt size (HR 0.18; 95% CI 0.04 – 0.81; P-value 0.01) and atrial septal aneurysm (HR 0.19; 95% CI 0.04 – 0.87; P-value 0.02) sub-groups.[163] Meta-analysis done by Agarwal S et al. [168], showed the rate of recurrent neurological events to be as high as 5 events per 100 patient years (95% CI: 3.6 to 6.9) in the medical management groups as compared to 0.8 (95% CI: 0.5 to 1.1) in the closure group, and the analysis revealed that elderly patients, patients with concomitant ASA and thrombophilia benefitted the most with device closure.

Despite the absence of statistical benefit in overall cohort in the randomized trial, these findings suggest that closure of PFO in patients with higher risk such as ASA or shunt size may be beneficial with respect to stroke risk. It remains to be seen whether benefit increases or reduces with a longer follow up period. Even though device closure is a promising alternative, presently, it can be potentially only used for patients who have a very high likelihood of an embolic event. Insertion of devices such as pacemaker or ICD may seem to add to the risk of thromboembolic event, but prophylactic closure of PFO solely based on risk offered by intravenous device placement does not seem to offer any significant clinical benefit and is not recommended. These necessitate further studies centered on a specific patient population and longer follow up to find the patients who may benefit from the closure therapy. Furthermore, the value and usefulness of closure devices needs to be further ascertained before considering it as a viable alternative to the present medical management for patients at moderate or low risk, including those undergoing intravenous device implantation.

With respect to newer developments in this field, the introduction of a leadless pacemaker seems to bring a significant paradigm shift in the technology. Even though new developments are still undergoing and the short- and long-term results of this device have been satisfactory [169, 170], it remains to be seen whether leadless pacemakers may act as a nidus for any thrombogenic and subsequent embolic activity. In the absence of an intravenous component, the possibility is rare. Hopefully, it would give rest to the issues of stroke or thromboembolic events in patients undergoing device implantation.

CONCLUSION

Data associating risk of stroke in patients undergoing device implantation are mostly non-confirmatory, and independently associating devices (leads) with stroke/PE may be an overestimation. In the absence of definitive data, emphasis should be given to careful and thorough evaluation of underlying thrombotic/embolic risk factors for patients undergoing device implantation. It is highly plausible that the risk of thrombus formation followed by embolism is essentially multifactorial in origin, wherein two or more factors combine or work synergistically to increase the probability of future adverse events. This seems more likely, because even though numerous studies have attempted to find independent predictors, they have resulted in diverse outcomes. This further points to the importance of extensive investigation to rule out various causes of cryptogenic stroke or embolic stroke of uncertain origin. Different medical management studies have been inconclusive, while interventional PFO closure as the strategy to prevent future embolic events was not found to be superior to medical management in the overall population, even though it is possible that selected high risk patients may benefit from closure. Intravenous device placement alone does not justify closure of PFO to decrease embolic risk. Managing patients by risk stratification based on identified risk factor of stroke may be the best way until further evidence is obtained. Further, long term prospective studies may add valuable information for clinicians. As for stroke/thromboembolism risks, leadless pacemakers may prove to be beneficial for patients requiring device implantation.

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Chapter 79

STROKE

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1. OVERVIEWS

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Stroke is identified by the sudden occurrence of a nonconvulsive, focal neurologic deficit. The classic etiologies of the stroke are: thrombosis, embolism, and hemorrhage. The major types of stroke include: atherosclerotic thrombosis, lacunes, embolism, hypertensive hemorrhage, ruptured aneurysms and vascular malformations, among others (Victor and Ropper, 2001)

Among all the neurologic diseases of adult life, stroke ranks first in frequency and impact on disability. Stroke constitutes at least 50 percent of the neurologic disorders in a general hospital. Stroke, after ischemic heart disease, is the second-commonest single cause of death worldwide, with over 5 million deaths per year globally. Despite advances in acute care and secondary preventive strategies, stroke remains a major burden on health-care resources worldwide. Conventional therapeutic measures have failed to restore neurological function post stroke. However, cell-based neurorestorative strategy is an emerging therapeutic modality and there is evidence that this therapy provides therapeutic benefit for the treatment of stroke.

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1.1. Incidence

U.S. Census Bureau has forecasted the distribution of incident stroke cases for the years 2010 to 2050. Over these 40 years, the number of incident strokes is expected to more than double, with the majority of the increase among the elderly (age >75 years) and minority groups (particularly Hispanics) (Howard G and Goff DC, 2012).

1.2. Risk factors

Several factors are known to increase the likelihood of stroke. The most important of these are hypertension, heart disease, atrial fibrillation, diabetes mellitus, cigarette smoking, and hyperlipidemia. Others, such as systemic disease associated with a hyper coagulable state and the use of birth control pills, also contribute, but to a lesser degree (Victor and Ropper, 2001).

1.3. Pathophysiology

Cerebral infarction basically comprises two pathophysiologic processes, 1) a loss in the supply of oxygen and glucose secondary to vascular occlusions and, 2) an array of changes in cellular metabolism as a consequence of the collapse of energy-producing processes, with damage to cell membranes. Of potential therapeutic importance are the observations that some of the cellular processes leading to neuronal death are not irrevocable and may be reversed by early intervention, either restoration of blood flow or prevention of the influx of calcium into the cell (Victor and Ropper, 2001). In cerebral hemorrhage, the resulting hematoma within brain parenchyma triggers a series of adverse events causing secondary insults and severe neurological deficits. The pathological pathway of second injury includes cytotoxicity of blood, hypermetabolism, excitotoxicity, oxidative stress and inflammation (Aronowski and Zhao, 2011).

1.4. Treatment

1.4.1. Management of Acute Phase of Intracerebral Haemorrhage

Hemorrhagic strokes are relatively less common compared to ischemic strokes, with the vast majority of hemorrhages being intracerebral as opposed to subarachnoid. The definitive diagnosis of a hemorrhagic stroke is based on CT imaging of the brain. Acute care should focus on prompt identification of the cause of haemorrhage, minimizing the risk of hemorrhage expansion by controlling blood pressure and correcting any underlying coagulopathy, and obliterating vascular lesions with a high risk of rebleeding (Flower and Smith, 2011).

1.4.2. Management of Acute Phase of Ischaemic Stroke

Thrombolysis with tissue plasminogen activator is now a well-established treatment for acute ischemic stroke and is associated with significant improvements in outcomes. Tissue plasminogen activators (recombinant t-PA and streptokinase), when administered intravenously, convert plasminogen to plasmin, the latter being a proteolytic enzyme capable of hydrolyzing fibrin, fibrinogen, and other clotting proteins. The benefits of intravenous thrombolysis up to 4.5 hours are now firmly established. Intra-arterial thrombolysis has been used for decades but data on its efficacy and safety are limited. Novel methods of reperfusion, such as mechanical clot retrieval and stenting, have high recanalization rates with acceptable safety and may be considered in patients with mid- to large-sized artery occlusion (Livesay S, 2014; Kirkman M et al., 2014).

1.4.3. Secondary Prevention

Anti-Platelet Treatment

Due to the high rate of recurrence, in high risk-patients (e.g., patients affected by atherosclerotic vascular disease), long-term antiplatelet therapy has contributed to the significant decline in vascular event rates in patients with ischemic stroke or transient ischemic attack (Hong K, 2014). Current guidelines for prevention of secondary stroke recommend the broad use of antiplatelet therapy for patients with a history of non-cardioembolic stroke or TIA. As for primary prevention, indications are less accurate because the absolute benefits of aspirin in reducing the occurrence of vascular events are generally much lower than in secondary prevention (Pinto A et al., 2014). The common anti-platelet agents include aspirin, clopidogrel, prasugrel and ticagrelor.

Blood-Pressure-Lowering Treatment

High blood pressure in the acute cerebrovascular event is associated with poor outcome. Gradual, sustained lowering of blood pressure is recommended in all stroke patients, but care is needed, particularly in patients with carotid or vertebrobasilar occlusive disease. The ideal time to initiate lowering of blood pressure after stroke is uncertain, but it should be initiated prior to discharge from the hospital. The optimum blood pressure-lowering drugs depend on patient comorbidities. The combination of an angiotensin-converting enzyme inhibitor (or angiotensin II receptor antagonist) and calcium channel blocker (or diuretic in the elderly) may be preferable, because the latter drugs (ie, calcium channel blockers and diuretics) also reduce blood pressure variability. (Hankey GJ, 2014).

Statin Treatment

Statins is now recommended in secondary prevention after stroke or transient ischemic attacks to reduce the risk of a new stroke or major cardiovascular events. However, some issues about the extent of the benefit of statins in some stroke patients and the risk of cerebral hemorrhage remain uncertain (Ní Chróinín D et al., 2013). Statins are effective in decreasing the risk of secondary strokes despite an increase in the risk of brain hemorrhage. A significant benefit of statin therapy was observed in men and women, in aged patients, and possibly in patients with carotid artery stenosis. Intensive statin therapy lowering the LDL-cholesterol beyond the cut-off value of 1.8 mmol/L (70 mg/dl) seems to be more effective than less

intensive treatment and without an increased risk of side effects. Overall, statins are well tolerated (Laloux P, 2014).

Anticoagulant Treatment

In patients with nonvalvular atrial fibrillation, oral anticoagulation with the vitamin K antagonists acenocoumarol, phenprocoumon and warfarin reduces the risk of stroke by more than 50%, whereas single or double antiplatelet therapy is much less effective and sometimes associated with a similar bleeding risk as vitamin K antagonists (Mani and Lindhoff-Last, 2014). Besides bleeding, and intracranial haemorrhage in particular, INR (international normalization ratio) monitoring remains the largest drawback of vitamin K antagonists. In the last decade, oral agents have been developed that directly block the activity of thrombin (factor IIa), as well as drugs that directly inhibit activated factor X (Xa), which is the first compound in the final common pathway to the activation of thrombin. These agents have been approved for stroke prevention in atrial fibrillation patients. Advantages include, no need to monitor. These agents have a fast onset of action, but lack an established specific antidote (Verheugt FW, 2013).

Carotid Revascularization

Patients with recently symptomatic carotid territory transient ischaemic attack or non-disabling ischaemic stroke and ipsilateral 50–99% internal carotid artery stenosis (measured by two concordant non-invasive imaging modalities) who are fit and willing for surgery should be offered carotid endarterectomy as soon as possible, ideally within the first few days and up to 1 week after the ischaemic event and when the patient is clinically stable (Rerkasem K and Rothwell PM, 2011).

Carotid endarterectomy should be done by a surgeon with an audited perioperative morbidity and mortality of less than 5% (Bonati LH et al., 2012).

Carotid endarterectomy is more appropriate than carotid stenting for patients older than 70 years of age who are otherwise fit for surgery because stenting carries a higher short-term risk of stroke and death. Carotid stenting may be as safe as endarterectomy in patients less than 70 years of age.

Carotid stenting could also be considered for patients who are not candidates for carotid endarterectomy because of technical, anatomical, or medical reasons. Interventionists should have expertise in carotid procedures and an expected risk of periprocedural morbidity and mortality of less than 5% (Bonati LH et al., 2011).

2. PRECLINICAL NEURORESTORATIVE STUDIES

Liyan Qiao

2.1. Animal Model

2.1.1. Ischemic Animal Model

Models Requiring Craniotomy

This group of methods requires opening of the skull to allow direct access to the cerebral arteries. In most instances, this has involved small craniotomies that allow distal branches of

vessels such as the middle cerebral artery (MCA) to be ligated, clipped, or sealed by photothrombosis or electrocoagulation (Howells D et al., 2010). Although occlusion of the vessel is usually permanent, ligatures can be released, pneumatic cuffs deflated and even thrombotic lesions created by electrocoagulation or photothrombosis can recanalize to permit transient occlusion.

Models Not Requiring Craniotomy

This group of methods is to use intra-arterial access to occlude cerebral arteries, including embolic model, intraluminal suture middle cerebral artery occlusion (MCAO) model, photothrombosis model, and endothelin-1-induced stroke model et al. Among these models, MCAO is the most commonly used model in the investigation of stroke in rodents (Liu F et al., 2011). This model offers a simple and less traumatic surgical approach, lends itself more readily to the study of reperfusion and has been adapted for use in continuous magnetic resonance imaging, produces focal occlusion of a large cerebral artery as seen in human stroke, and can be done in a high-throughput manner.

Below is an example of MCAO model for rats (Zhang L, 2011). Animals were anesthetized with 3.5% isoflurane and maintained with 1.0% to 2.0% isoflurane in 70% N₂O and 30% O₂ using a face mask. Rectal temperature was maintained at 37°C throughout the surgical procedure using a feedback-regulated water heating system. The right common carotid artery, external carotid artery, and internal carotid artery were exposed. A 4-0 monofilament nylon suture (18.5 to 19.5 mm), determined by the animal weight, with its tip rounded by heating near a flame, was advanced from the external carotid artery into the lumen of the internal carotid artery until it blocked the origin of the MCA. Two hours after MCAO, animals were reanesthetized with isoflurane and reperfusion was achieved by withdrawal of the suture until the tip cleared the lumen of the internal carotid artery.

2.1.2. Intracerebral Hemorrhage (ICH) Model

Three major ICH models have been developed: (1) single blood injection, (2) microballoon-induced formation of ICH, (3) collagenase injection.

Single blood injection commonly involves the intracerebral injection of autologous blood, which is a straightforward and effective technique for producing ICH model. This type of model has been developed in large animals (e.g., dogs, cats, pigs and monkeys), by injecting blood into the frontal lobe. For small animals (e.g., rats and mice) blood is injected into the caudate. This method does not reproduce the arterial vessel rupture present in human spontaneous ICH, but it does control the volume of blood injected and has been shown to be useful for the study of pathophysiological and biochemical consequences of ICH.

Micro balloon insertion was developed as a way to study the mass effects of ICH. A micro balloon was mounted on a no. 25 blunted needle and inserted stereotactically into the right caudate nucleus. The burr hole was then sealed with dental cement. After a 30-min stabilization period, the balloon was inflated to 50 µl over a period of 20 s with radiopaque contrast medium. In the sham-treated control group, the balloon was inserted but not inflated. Brain edema formation was successfully produced, much as occurs in the presence of any intracerebral mass, but the potentially significant effects of the presence of blood and clot in ICH cannot be addressed by this method (Manaenko A, 2011).

Collagenase injection model of ICH involves the injection of bacterial collagenase into brain. Originally developed in rats, this approach has also been used extensively in mice.

Collagenase dissolves the extracellular matrix, ultimately leading to blood vessel rupture and ICH. This model mimics the vascular disruption in spontaneous human ICH, but also induces widespread disruption of the extracellular matrix which is not found in spontaneous cases of human ICH (Manaenko A, 2011).

Below is an example of the ICH mouse model induced by injecting collagenase into the striatum (Xi G et al., 2013). Mice were anesthetized with isoflurane (1.8%) and placed in a stereotaxic frame. A burr hole was made and a 30-gauge needle was inserted into the striatum (location 2.5mm lateral to the midline, 0mm to bregma, 3.5 mm depth below the skull). Half a microliter of saline containing 0.06 IU of collagenase was injected over 5 min using a micropump. After infusion, the needle was left in place for 25min, and slowly removed; the skin incision was sutured. Rectal temperature was maintained at 37.5°C with a heating pad during the surgery. Mice were kept in an incubator to maintain body temperature for 3h after the surgery.

2.2. Neurorestorative Strategies

2.2.1. Medicine and Factors

Following cerebral ischemia, a complicated cascade of biochemical events occurs, ultimately leading to the death of neurons. Within this cascade, many molecular targets can be pharmacologically modulated to produce neuroprotection. The potential targets include glutamate release, glutamate receptor activation, excitotoxicity, calcium influx into cells, mitochondrial dysfunction, activation of many intracellular enzymes, free radical production, nitric oxide production, apoptosis, and inflammation, among others.

Heat Shock Protein (Hsp70)

Preliminary treatment with Hsp70 significantly reduced the ischemic zone. Long-term treatment of the ischemic rats with Hsp70 formulated in alginate granules with retarded release of protein further reduced the infarct volume in the brain as well as apoptotic area. The members of the HSP70 family work together as networks to facilitate organelle communication and regulate inflammatory signaling and cell survival after cerebral ischemia (Shevtsov M et al., 2014).

Estrogens

The potency and efficacy of estrogens for the treatments of ischemia and reperfusion injury, have been well demonstrated for a variety of cell types in the neurovascular unit (Koellhoffer and McCullough, 2013). The effects of estrogens on cerebral vasculature homeostasis and endothelium may reduce disruption of the blood brain barrier (BBB) induced by ischemic stroke (Liu and Yang, 2013).

Voltage-Gated Cation Channel Modulators

One approach to provide therapeutic benefit following ischemic stroke has been to target neuronal voltage-gated cation channels, and particularly blockers of calcium and sodium channels, for post-stroke neuroprotection (Gribkoff and Winquist, 2005). Large-conductance calcium- and voltage-dependent potassium channels (maxi-K channels), which hyperpolarize

ischaemic neurons, reduce excitatory amino acid release, and reduce ischaemic calcium entry (Gribkoff and Winquist, 2005).

NMDA Antagonists

N-Methyl-D-aspartate (NMDA) antagonists which have been investigated in stroke include: aptiganel hydrochloride, dextrophan, dextromethorphan, and magnesium ion (Ogden and Traynelis, 2011). Selective NMDA antagonists such as ifenprodil and eliprodil have demonstrated preclinical neuroprotective efficacy (Kalia L et al., 2008). However, recent findings of a persistent post-stroke decline in NMDA receptor density, which plays a pivotal role in plasticity and memory formation, suggest that NMDA receptor stimulation, rather than inhibition, may prove beneficial in the subacute period after stroke. The beneficial role for NMDA receptor stimulation during the recovery period after stroke is most likely due to enhanced neuroplasticity rather than neuroprotection (Dhawan J et al., 2011).

Radical Scavengers

Radical scavengers (such as vitamin E, sulfhydryl compounds and nitron spin traps) have been studied in stroke, which can promote detoxification of ROS (superoxide dismutase, catalase or peroxidase conjugates), and prevent radical generation. The use of NXY-059 has been associated with clinical benefits in animal models of stroke, and was associated with a significant improvement in the functional recovery (Green and Ashwood, 2005).

Ebselen was shown to attenuate oxidative DNA damage and enhances its repair activity in the thalamus after focal cortical infarction in hypertensive rats (He M et al., 2007). A subsequent study has shown that Ebselen provided protection against delayed neuronal death and oxidative damage from focal ischemia in hypertensive rats when administered 24 h post-stroke (Rawan and James, 2010).

Wu et al., (2014) systematically reviewed the efficacy of edaravone in animal models of focal ischemia. They identified 49 experiments describing edaravone outcome in 814 animals; 30 experiments (519 animals) reported functional benefit and 35 experiments (503 animals) reported improvement in structural outcome. Edaravone improved functional and structural outcome by 30% (95% confidence interval 23-37%) and 25% (95% confidence interval, 21-29%), respectively.

Statins

Continuous oral administration (avoiding withdrawal) with statins after stroke may reduce the extent of post-ischemic brain damage and improve neurological outcome in rats (Saito T, 2014).

2.2.2. Bioengineering

Stimulation of cerebral angiogenesis by gene delivery is under extensive investigation. (Kurinami H et al., 2014). Vascular endothelial growth factor (VEGF) enhances neurogenesis in ischemic brains. Li et al., (2009) transferred the VEGF gene into brain tissue with recombinant adeno-associated virus serotype 1 (rAAV1) vectors and VEGF expression reduced the size of cerebral infarction and improved neurological function. It also enhanced the proliferation of neural progenitor cells in the subventricular zone and promoted their migration to the ischemic lesion. Neural precursors in the subgranular zone of the dentate

gyrus were also increased; however, most of these cells did not move to the ischemic lesion and integrated within their region of origin.

Endothelial cell proliferation and the number of newly formed microvessels were greatly increased in the MicroRNA-210 (miR-210)-mice (Zeng L et al., 2014).

Gene-Modified Stem Cells

Keeney et al. (2013) described a recently developed strategy for stimulating vascular growth by programming stem cells to overexpress angiogenic factors in situ using biodegradable polymeric nanoparticles. The strategy utilized stem cells as delivery vehicles by taking advantage of their ability to migrate toward ischemic tissues in vivo. Using the optimized polymeric vectors, adipose-derived stromal cells were modified to overexpress an angiogenic gene encoding VEGF in a mice hindlimb ischemia model. Prior to Keeney's work, Cho et al. (Cho et al., 2012) developed nanoparticles as vehicles to genetically modify human umbilical vein endothelial cells with VEGF. In a mouse model of hindlimb ischemia, VEGF nanoparticle transfection promoted engraftment of umbilical vein endothelial cells into mouse vasculature as well as survival of transplanted cells in ischemic tissues.

Bone marrow mesenchymal stromal cells transfected with a vector encoding human Notch1 intracellular domain was shown to be effective for the treatment of a chronic stroke model, exhibiting equal or superior immunosuppressive and angiogenic activity to parental MSC in vitro, and producing extracellular matrix that is exceptionally supportive for neural cell growth (Aizman et al., 2014).

2.2.3. Cell Therapy

Granulocyte-Colony Stimulating Factor (G-CSF)

Broadly speaking, clinical cell-based neurorestorative therapy can be divided into "endogenous" and "exogenous" approaches: The endogenous approach aims to stimulate mobilization of stem/progenitor cells already present within the individual. G-CSF is one of the successful examples for endogenous approaches. G-CSF is used to mobilize hematopoietic stem cells into the peripheral blood.

However, G-CSF has also been shown to have neuroprotective properties beyond simply cell mobilization, and reduces infarct volume in experimental cerebral ischemia (England T, et al. 2009). In the acute phase of cerebral ischemia, the neuroprotective mechanism of G-CSF includes inhibition of glutamate release, reduction of inflammation, antiapoptotic activity, and suppression of edema formation (Nomani F et al., 2013). Further, G-CSF has a direct protective effect against glutamate-induced excitotoxicity in cultured neurons (Pan C et al., 2010).

Embryonic Stem Cells (ESCs)

Daadi et al. (2009) transplanted NSPCs derived from ESCs into the post stroke rat brain, and showed that transplanted cells could differentiate into neurons, oligodendrocytes, and astrocytes. Neural progenitor cells derived from murine or monkey ESCs were also reported to survive in stroke lesions of brain, and differentiated into mature neurons (Buhnenmann et al., 2006; Hayashi et al., 2006).

These results indicate that ESCs are a promising cell source, but human fertilized eggs are needed to establish each ESCs. This ethical problem interferes with the clinical application of ESCs. Risk of tumorigenesis is a major obstacle to human embryonic and induced pluripotent stem cell therapy (Seminatore C et al., 2010).

Induced Pluripotent Stem Cells (iPSCs)

Many researchers have reported that hiPS cells could be differentiated into various kinds of neurons including glutaminergic (Qiang et al., 2011), motor (Ebert et al., 2009), and dopaminergic (Caiazzo et al., 2011) neurons. After human neural precursor cells (hNPCs) derived from iPS cells were transplanted into the murine brain they survives as mature neurons (Chen et al., 2010).

It has been shown that grafted human iPSCs survive, differentiate to neurons and ameliorate functional deficits in ischemic stroke aged brain (Tatarishvili J et al., 2014; Yuan T et al., 2013; Chen SJ et al., 2010). In addition, human skin-derived iPSCs can be specifically differentiated into cortical neuronal progenitors, which survive, differentiate to functional neurons and improve neurological outcome after intracortical implantation in a rat stroke model (Tornerio D et al., 2013). Transplantation of iPSCs also contributes the improvement of neurological function in experimental intracerebral hemorrhage rats (Qin J et al., 2013). iPSC-derived NPCs are effective cells for the treatment of stroke. The transplanted animals exhibited significant reduction of stroke-induced inflammatory response, gliosis and apoptosis, and increased endogenous neurogenesis (Chang D et al., 2013; Polentes J et al., 2012). iPSCs have ability to form new functional neurons which accompany functional recovery after stroke (Oki K et al., 2012).

Recently, the creation of vector-free and transgene-free human iPSCs provides a new generation of stem cells with a reduced risk of tumor formation that was associated with the random integration of viral vectors seen with previous techniques. The new kind of iPSCs has been shown to be safe and efficacious in enhancing recovery after focal ischemic stroke in mice (Mohamad O et al., 2013).

Neural Stem /Progenitor Cells (NSPCs)

NSPCs could survive and migrate towards the lesion in rats with an ischemic stroke following intracisternal administration. Electron microscopy examination also suggested that the transplanted cells showed signs of neuronal differentiation (Zhang ZG et al., 2003).

Immortalized Cell Lines: NT2 Cell Line

The immortalized cell line NTera-2 (NT2) was derived from a human teratocarcinoma over 20 years ago (Arsenijevic Y et al., 2001) After several weeks of exposure to retinoic acid and mitotic inhibitors, these cells differentiate into post-mitotic neuron-like cells, named NT2N cells which display a variety of neuronal features (Andrews PW et al., 1984). These cells maintain their neuronal phenotype both in vitro and in vivo for over a year, without reverting to a neoplastic state (Pleasure SJ et al., 1993).

The transplantation of NT2N cells into the ipsilateral striatum of ischemic rat brains could improve functional recovery, with some parameters of behavioral improvement persisting for up to 6 months post-transplantation. Moreover, no evidence of tumorigenicity was found following post-mortem examination of the rats (Banerjee S, 2011).

Mesenchymal Stromal Cells (MSCs)

Transplanted MSCs aggressively can migrate toward the damaged CNS tissue, promote the recovery of motor function after cerebral infarct, rescue the host neurons (Kuroda et al., 2011; Uccelli et al., 2008) and ameliorate neuronal injury (Hokari et al., 2008). MSCs also improve cognitive function due to chronic cerebral ischemia (Shichinohe et al., 2010). To evaluate the quality of preclinical evidence for MSC treatment of ischemic stroke, a meta-analysis showed that 44 /46 studies reported that MSCs significantly improved outcome (Vu et al., 2014).

Umbilical Cord Blood Cells (UCBCs) and Related Cells

Nonfractionated human umbilical cord blood (UCB) (Vandrome et al., 2004) or mononuclear UCB cell fraction (Boltze et al., 2012), have been employed to treat experimental stroke in the laboratory, evoking significant behavioral and anatomical recovery after transplantation. Intraventricular transplantation of UCB-derived mesenchymal stromal cells also significantly attenuated posthemorrhagic hydrocephalus and brain injury after intraventricular hemorrhage (Ahn S et al., 2013).

Iskander et al. using MRI, showed that after intravenous administration of hUCB -derived endothelial progenitor cells in acute rat stroke model, these administered cells accumulated in stroke-affected hemispheres and reduced the infarcted volume (Iskander et al., 2013); however, in another study, most administered cells were found to traffic to the lungs immediately following IV administration and approximately 1% of total administered dose locate at the site of injury (Arbab et al., 2012).

A single injection may be sufficient for effective treatment of stroke with umbilical cord cells. And multiple injections were not superior to single injection therapy in both functional outcome and histological assessments in a stroke model (Shehadah et al., 2013). However, a dose dependent relationship has been shown between therapeutic effect and human umbilical tissue cell number (Seghatoleslam et al., 2013).

Zhang et al. (2013) investigated the effect of UCB cells on aged rats after embolic stroke. Intravenous administration of UCB cells significantly improved neurological functional recovery, increased synaptogenesis and vessel density in the ischemic boundary zone, increased progenitor cell proliferation in the subventricular zone without reducing infarct volume in aged rats.

The same group (Zhang et al., 2012) also tested the efficacy of different administer route, including intraarterial (IA), intravenous (IV), intra-cisterna magna (ICM), lumbar intrathecal (IT), or intracerebral injection (IC).

While IA administration resulted in the highest donor cell number detected within the ischemic brain compared to the other routes, umbilical tissue cells treatments via all routes can provide therapeutic benefit after stroke.

IA, IV, IC seem to bring enhanced benefits due to their ability to increase synaptophysin immunoreactivity and to reduce in apoptotic cells. Transplantation of cryopreserved UCB mononuclear cells does not induce sustained recovery after experimental stroke in spontaneously hypertensive rats (Weise et al., 2014).

3. CLINICAL NEURORESTORATIVE STUDIES

Liyan Qiao, Lin Chen, Hongyun Huang

3.1. Scoring System of Assessment for Stroke

Stroke scales can be classified as clinicometric scales and functional impairment, handicap scales. The severity of the stroke can be assessed by scoring system, such as the National Institutes of Health Stroke Scale (NIHSS) (Brott T, 1989). Despite advantages of modified NIHSS and Scandinavian Stroke Scale comparing to the NIHSS, including their simplification and less inter-rater variability; most of the stroke neurologists around the world continue using the NIHSS (Ghandehari K, 2013). The modified Rankin scale (mRS) and Barthel index (BI) are widely used as functional impairment and disability scales.

3.2. Neurorestorative Strategies

3.2.1. Medicines and Molecules

Many of treatments have been shown to provide neuroprotection in pre-clinical studies. However, this success in the laboratory has not been translated to the clinic, and currently there are no approved neuroprotective or neurorestorative treatments for stroke.

Radical Scavengers

Ebselen has shown some therapeutic promise in patients with acute ischemic stroke in an initial clinical study (Ogawa A et al., 1999). Edaravone has also shown promise in some clinical trials for ischemic stroke. Ishibashi (Ishibashi A et al., 2013) et al. investigated the effect of the administration of edaravone at the acute stage of ischemic stroke. Of the 625 patients enrolled in the study, 237 (37.0%) received both edaravone and conventional treatment, while the other 388 (62.0%) patients underwent conventional treatment only. The administration of edaravone did not have a significant effect on total death from all types of cerebral infarction. However, treatment with edaravone showed a favorable tendency compared to conventional treatment after adjustments for age and gender. Edaravone has been used in patients with acute ischemic stroke in Japan for over 10 years but does not have marketing authorization in Europe or America (Wu S et al., 2014).

Calcium Antagonists

In a meta-analysis of calcium antagonists for acute ischemic stroke, 34 trials were included. No evidence is available demonstrating that calcium antagonists in patients with acute ischemic stroke are effective. In addition, comparisons of different doses of nimodipine suggested that the highest doses were associated with worse outcome (Zhang J, 2012).

NMDA Antagonists

The use of NMDA antagonists was associated with side effects and no clear clinical benefit in clinical trials (Muir K, 2006). Two preliminary trials of intravenous magnesium

show no evidence of adverse effects. In a Phase 2 trial of Selfotel in acute stroke patients, a dose-dependent toxicity was reported (Grotta J et al., 1995).

Scavenging Divalent Metal Ions

DP-b99 seemed well tolerated by patients with acute stroke, and a Phase IIb trial reported better outcomes among DP-b99-treated patients than a control group on a secondary outcome measure (Rosenberg G et al., 2011). However, in a randomized, double-blind, placebo-controlled, multicenter, parallel-group trial of intravenous DP-b99 administered in ischemic stroke patients, DP-b99 shows no evidence of efficacy in treating acute human ischemic stroke (Lees KR et al., 2013).

Minocycline

Minocycline, in small randomized controlled human trials, is a promising neuroprotective agent in acute stroke. However, a large study has not been powered to reliably identify or to exclude a modest but clinically important treatment effect of minocycline (Kohler E, 2013).

3.2.2. Cell Therapy

Cell therapy clinical trials confirmed the safety and feasibility of some kinds of cell therapy in stroke patients (Kalladka D et al., 2014). Cell therapy involves the delivery of cells locally (eg, direct intracerebral implantation), intrathecally or systemically (eg, intravenous or intra-arterial).

G-CSF has been shown to be safe in Phase I clinical trials of human stroke when used within 7 days (Shyu WC et al., 2006; Zhang JJ et al., 2006) or 7-30 days post-stroke (Sprigg N et al., 2006). Low-dose G-CSF (150 and 300 µg/body/day) was safe and well tolerated in acute and ischemic stroke patients (Moriya Y, et al, 2013). Another randomized, double-blind, placebo-controlled trial suggests that a higher dose of G-CSF (10µg/kg/day) is safe when administered in subacute stroke (England TJ et al., 2012).

However, although there was a trend for reduced infarct growth, G-CSF treatment failed to show efficacy in functional evaluation in a large Phase 2 trial in acute ischemic stroke patients (Ringelstein EB et al., 2013). Compare to GSF, exogenous cell therapy is the main focus for developing new treatment for stroke.

3.2.2.1. Bone Marrow Mononuclear and Stromal cells (BMMNCs and BMSCs)

BMMNCs and BM-BMCs in Acute/Subacute Stage of Stroke

Intra-arterial autologous BMMNCs transplantation was performed for acute ischemic stroke (Mendonça et al., 2006; Moniche F et al., 2012; Friedrich M et al., 2012). Patients demonstrated a partial improvement and a slight decrease in the ischemic area by MRI and hypoperfusion by SPECT, even for patients with moderate to severe acute middle-cerebral-artery strokes. Cell therapy is also safe, feasible and effective via an intravenous route of administration (Savitz S et al., 2011) and for patients with subacute ischemic stroke (Honmou O et al., 2011).

BMMNCs and BMSCs in Chronic Stage Of Stroke

An early tissue distribution of BMMNCs was reported in a chronic stroke patient after intra-arterial delivery (Barbosa da Fonseca LM, 2009). Part of the cell suspension was radio-labeled with ^{99m}Tc to monitor the fate of transplanted BMMNCs. Brain SPECT views revealed uptake and retention of the labeled BMMNCs in the territory of the left MCA for up to 48 hours. The remaining uptake occurred mainly in the liver and spleen. This distribution is consistent with Correa's report (Correa PL, 2007).

Autologous BMMSCs were injected intravenously into 12 patients with stroke. Serial evaluations showed no severe adverse cell-related effects and a trend of functional recovery (Honmou O, 2013) and similar results were reported by intravenous cell transplantation (Bringas ML et al., 2011; Alvarez-González et al., 2009) and also by intra-arterial cell transplantation (Battistella et al., 2011). Long-term follow-up is, however, required to provide convincing evidence for its safety (Lee J et al., 2010). Additional studies are in underway (Kim S et al., 2013).

3.2.2.2. Neurons Derived from Teratocarcinoma

Kondziolka et al. (Kondziolka D et al., 2000) studied the safety and feasibility of intraparenchymal transplantation of neuronal cells derived from human teratocarcinoma in twelve patients with basal ganglia stroke and fixed motor deficits. No adverse effects were observed during a 12-month follow-up.

The European Stroke Scale score improved in six patients, and six of the eleven PET-scans performed six months after the procedure showed increased FDG uptake at the site of implantation. Nelson et al. (Nelson PT et al., 2002) reported the first postmortem brain findings for a patient 27 months after transplantation, who died of acute myocardial infarction. Neurofilament immunoreactive neurons were identified in the graft site, and fluorescent in situ hybridization revealed polyploidy in groups of cells at this site, and there was no evidence of a neoplasm after more than 2 years implantation. In a Phase 1 human trial, Kondziolka et al. demonstrated that (Kondziolka D et al., 2005), neuronal transplantation is feasible, safe and effective. In the following Phase 2 randomized clinical trial for human neuron transplantation combined with rehabilitation program, improvement of measurable movement and daily living activities were observed in some patients (Kondziolka D et al., 2005).

3.2.2.3. Umbilical Cord Stromal Cells

Umbilical cord stromal cells have been shown as a feasible and safe approach for the treatment of ischemic stroke with potential to improve the neurological (Jiang Y et al., 2013).

3.2.2.4. Autologous Peripheral Blood Stromal Cells

After G-CSF mobilization, intracerebral implantation of autologous peripheral blood stem cells has been shown safe, feasible and effective in stroke patients in a randomized phase II study (Chen DC et al., 2014). Wang L et al. indicated administration of G-CSF-mobilized autologous CD34 positive cells was safe in patients post stroke (Wang L et al., 2013).

Other Cell Type

Neurotransplantation of fetal porcine cells in patients with basal ganglia infarcts was demonstrated as safe and feasible in a preliminary study (Savitz S et al., 2005). Two patients showed improvement in speech, language, and/or motor impairments which persisted at 4 years.

Combination Cell Therapy

Bahasin A et al. (Bahasin A, 2013) recruited forty chronic stroke patients with treatment with autologous mononuclear and mesenchymal stromal cells intravenously. The safety test profile was normal with no mortality or cell related adverse reactions in patients, and statistical significant improvement by assessment of modified Barthel Index and an increased number of cluster activation in Brodmann areas BA 4, BA 6 in EEG was evident. Cell suspension consisting of cells from immature nervous and hemopoietic tissues was subarachnoidally transplanted to 10 stroke patients. The results showed significantly functional improvement six months after treatment (Rabinovich SS et al., 2005). A total of 10 consecutive stroke patients were treated by combination cell transplantation including olfactory ensheathing cells, neural progenitor cells, umbilical cord mesenchymal cells, and Schwann cells. After 6 months to 2 years follow up, the patients achieved different degrees of neurological function amelioration including improved speech, muscle strength, muscular tension, balance, pain, and breathing; most patients had an increased Barthel index score and Clinic Neurologic Impairment Scale score (Chen L et al., 2013).

3.2.3. Neuromodulation

The effects of repetitive transcranial magnetic stimulation (rTMS) on motor and sensory function recovery in stroke patients have been investigated. For hand motor function recovery after stroke, rTMS of the lesioned or contralesional motor cortex was combined with motor training and showed ambiguous effects; some patients improved, whereas others did not show any rTMS effect (Le Q et al., 2014). Central post stroke pain (CPSP) is one of the most refractory chronic pain syndromes. The rTMS of the primary motor cortex has been demonstrated to provide moderate pain relief for CPSP. The mechanism underlying the pain relief is due to restoration of abnormal cortical excitability (Hosomi K et al., 2013).

Transcranial direct current stimulation (tDCS), a non-invasive neuromodulation technique inducing prolonged brain excitability changes and promoting cerebral plasticity, is a promising option for neurorehabilitation, such as limb function and aphasia (JMonti A et al., 2013). The effects of rTMS/tDCS on the functional architecture of the motor system depend upon lesion location, degree of impairment and number of treatment sessions. Furthermore, analyses of regional brain activity and motor network connectivity allow prediction of the behavioral effects of brain stimulation (Grefkes C et al., 2012).

Robotic treatment is effective to reduce motor impairment in chronic stroke patients (Mazzoleni S et al., 2013). An electromyography (EMG)-driven hand robot developed for post-stroke rehabilitation training showed significant motor, spasticity and muscle coordination improvements in the hand (Hu XL et al., 2013). Another Hand Exoskeleton Rehabilitation Robot had benefits in range of motion, grip strength in the hands of post stroke patients (Godfrey SB et al., 2013). Robot-aided gait training could provide selective control on one of the essential subtasks of walking (B et al., 2013). In a prospective, multicentre,

parallel-group randomized trial enrolled patients with motor impairment for more than 6 months and moderate-to-severe arm paresis after a cerebrovascular accident in Switzerland; 38 patients assigned to robotic therapy had greater improvements in motor function in the affected arm over the course of the study. However, the authors pointed out that absolute difference between effects of robotic and conventional therapy was small and of weak significance (Klamroth-Marganska V et al., 2014). A tetraplegia patient with brainstem stroke could perform three-dimensional reaches and grasp movements using a neural interface system-based control of a robotic arm. Although robotic reach and grasp actions were not as fast or accurate as those of an able-bodied person, neural interface system is one of the potential treatments for disability caused by stroke (Hochberg L et al., 2012).

In conclusion, although there are major advances in understanding of the pathophysiology of cerebral ischemia, therapeutic options for acute ischemic stroke remain very limited. Consequently, successful treatment of acute ischemic stroke remains one of the major challenges in clinical practice. For the sequelae /of chronic stage of stroke, few of the treatment options have proven efficacious in clinical studies despite tremendous progress in preclinical studies. Cell-based neurorestorative strategies possibly offer the broadest range of potential treatments, because of multiple therapeutic options, such as cell types, routes of administration, therapeutic windows, and combination with other strategies. Cell therapy for stroke has demonstrated efficacy in the improvement of neurological outcome and quality of life of patients in the acute or chronic stage of stroke. Further investment in cell-based therapy for the neurorestorative treatment of stroke is therefore warranted.

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Chapter 80

INNOVATIONS IN STROKE REHABILITATION: FROM RESEARCH TO CLINICAL PRACTICE

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ABSTRACT

Stroke represents the leading cause of disability among the elderly in the industrialized world. Indeed, no more than the 40% of the stroke survivors is able to walk independently, and/or to properly use the upper limb, and only after having performed an appropriate rehabilitative treatment. Moreover, post-stroke cognitive deficits, including aphasia, apraxia and neglect, and other neuropsychiatric abnormalities may negatively impact on patients' quality of life.

One key principle of post-stroke neurorehabilitation is the repetitive creation of specific learning situations to promote mechanisms of neural plasticity in stroke recovery. Indeed, there is growing evidence of achieving a better outcome of neurorehabilitation with early initiation of treatment, high intensity, with specific goals and active therapies, and the coordinated work and multimodality of a specialized team.

To this end, robotic neurorehabilitation has the potential for a greater impact on impairment due to easy deployment, its applicability across of a wide range of motor

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disability, its high measurement reliability, and the capacity to deliver high dosage and high intensity training protocols.

Aim of this chapter is to provide a comprehensive overview on the potential role of assistive technology in post-stroke rehabilitation, focusing either on robotic gait and upper limb neurorehabilitation or computerized cognitive rehabilitation. Emerging and promising tools, including telerehabilitation and Computer Assisted Rehabilitation Environment (CAREN), will be also discussed.

1. INTRODUCTION

Stroke represents the leading cause of disability among adults in the industrialized world. Although data trends indicate that stroke-related death rate is declining, the physical and psychological impacts following a stroke can be devastating [1, 2]. Indeed, even though about 80% of individuals with stroke survive to the acute event, only about 40% is able to walk independently, but only if they have performed an appropriate rehabilitative treatment [3]. The most common feature in patients affected by stroke is hemiparesis with spasticity, which is responsible of the typical postural pattern, with an asymmetric posture and a reduced weight-bearing on the paretic limb, severe balance dysfunctions, and an increased risk of falling [4]. Interestingly, up to 85% of the patients show an initial deficit in the arm, and three to six months later, problems remain in 55–75% of the patients[5]. Moreover, oropharyngeal dysphagia occurs in 45-65% of patients after acute stroke, being the most important risk factor for the development of pneumonia, which in turn accounts for about 30% of all stroke-related deaths[6].

Finally, about one-fifth of stroke survivors may experience mood and anxiety disorders [7], leading to worse functional outcomes, whereas approximately one-quarter of these patients may develop dementia [8]. Other cognitive impairments, including attention, language, and executive function impairments occur in around 50% of stroke survivors [9]. The need of an appropriate rehabilitative approach to the treatment of motor disorders is widely recognized, since the type of training strategy adopted can significantly influence the degree of motor recovery [10]. Moreover, cognitive rehabilitation and a proper treatment of post-stroke depression may significantly improve patient's training participation, and thus functional outcomes [11].

Recovery from a stroke event is a complex process, likely occurring through a combination of spontaneous and learning mediated processes, whose knowledge is helpful in determining when to expect recovery and for planning appropriate treatment and timing of rehabilitation [12]. Although it is widely recognized that most spontaneous behavioral recovery tends to occur within the first 3 months after stroke onset, different patterns of recovery, depending on many complex factors, may extend this period [13]. Indeed, cerebral plasticity may be present also in chronic stroke patients, as evaluated by transcranial magnetic stimulation and advanced neuroimaging techniques [14].

Patients' improvement after rehabilitation increases with the training intensity and is related mainly to the tasks practiced during therapy. To this end, robotic neurorehabilitation has the potential for a greater impact on impairment due to easy deployment, its applicability across of a wide range of motor impairment, its high measurement reliability, and the capacity to deliver high dosage and high intensity training protocols.

2. ROBOTIC VERTICALIZATION

Although physical therapy may be beneficial in limiting the effects of bedridden condition and stroke medical complications and facilitating maximal functional recovery, mobilizing severely impaired or non-cooperating patients is often unprofitable. In order to improve and standardize physiotherapy-assisted VT (pVT), several robotic devices have been developed. In particular, the ERIGO device (Hocoma AG, Volketswil, Switzerland) combines progressive verticalization, cyclic leg movement (which allows stepping reinforcement in combination with step synchronized muscle functional electrical stimulation -FES- at lower limb), and body weight loading to ensure the safe stabilization in the upright position of the patient).



Figure 1. A patient affected by severe stroke during Erigo training.

FES combined with passive stepping movements may be an effective modality to increase circulating blood volume and thereby tolerance to postural hypotension in healthy subjects. Notably, FES stepping has been shown to be an effective tool for muscle strengthening and walking improvement in neurological patients, including hemiplegic ones [15-17]. Indeed, robotic verticalization (rVT) shows many advantages in comparison to pVT, e.g., the body weight loading and the possibility to continuously perform cyclic leg movements in addition to FES. More in detail, rVT maximizes the potential for longitudinal weight bearing through the lower extremities in a position of hip-extension/knee-extension/ankle-dorsiflexion, which is difficultly obtained in the pVT setting. Moreover, rVT allows strengthen exercises of body weight shifting from one leg to the other, which are not

simply carried out in severe post-stroke patients. Nevertheless, the presence of the physiotherapist is always essential, since patient's cooperation has to be constantly ensured and stimulated [18-19].

3. ROBOTIC GAIT TRAINING

Over the past years, beyond conventional physical treatment, a variety of new rehabilitative strategies, including gait training on treadmill with body weight support (BWS), have been carried out, demonstrating their effectiveness in improving walking performances [20]. A recent systematic review provides evidence that the use of electromechanical-assisted gait training devices in combination with physiotherapy increases the chance of regaining independent walking ability for people after stroke. Such effectiveness has been mainly demonstrated concerning the acute and subacute post-stroke phase, but the role of robotic-assisted gait rehabilitation in chronic stroke is still underdebate [21].

Two groups of robotic-assisted devices were developed: end-effector type and exoskeleton type (9-11) [22].

3.a. End-effector Devices

End-effector devices work by applying mechanical forces to the distal segments of limbs whereas exoskeleton-type devices have robotic arms aligned with the anatomical axes of the subject thus controlling proximal and distal joints. The end-effector type includes mainly the "*Gait Trainer*" (GT) (Reha-Stim; Berlin, Germany) and the "LokoHelp" LokoHelp group; Weil am Rhein, Germany. The GT includes a system for BWS and two footplates to which the patient's feet are fixed by straps. The plates are positioned on two bars, two rockers and two cranks that provide the propulsion thus simulate gait like movements. A recent study has demonstrated that GT-BWS walking training might be safer than overground walking training in subacute stroke patients, as evidenced by less patient's cardiometabolic demand.

The "LokoHelp" device is fixed onto the band of the treadmill and transmit the treadmill movements to levers positioned on both side of the device.

3.b Lokomat

The prototype of the exoskeleton type is the Lokomat (Hocoma; Zurich, Switzerland). The Lokomat is the most advanced robotic technology available for gait training and neurological re-patterning. While suspended above a treadmill in a secure and stabilizing harness, the user's legs are secured to the Lokomat's robotic legs. The controlled motion of the robotic legs guides the user's legs in an efficient and effective gait pattern. This motion, in addition to weight bearing through the legs, stimulates repatterning of the central nervous system. Speed, ROM, body weight support and guidance are all controlled by the therapist through an advanced software, and the Lokomat charts user progress with measurements for stiffness, strength, range of motion and distance. Compared to *Lokomat Nanos*, the newer

Lokomat-Pro exercises in a virtual reality environment with a constant audio and visual feedback (Figure 2).



Figure 2. Lokomat-Pro gait training in our Research Neurorehabilitation Laboratory.

Several controlled trials showed a superior effect of Lokomat in acute stroke patients with respect to walking ability and velocity as patients walk more symmetrically, with higher speed and a more efficient gait [23-25].

Kelley et al. [26] in their blinded randomized clinical trial have found no significant differences in gait velocity, endurance, or functional level between the two groups of chronic stroke survivors undergoing either Lokomat or traditional over-ground gait training.

Moreover, Hornsby et al. [27] showed greater improvements in speed and single limb stance time on the impaired leg in those subjects who received therapist-assisted locomotor training, with an interesting improvement on the perceived rating of the effects of physical limitations on quality of life only in individuals with severe gait deficits. On the contrary, a recent study by Ucar et al. [28] has demonstrated a significantly improvement on walking speed in patients affected by post-stroke chronic hemiplegia undergoing Lokomat training than those performing traditional training.

We have recently demonstrated that Lokomat training may improve mood, coping style and, maybe, also cognitive performance beyond the motor functional status. We have indeed postulated that the positive impact of LOKOMAT on mood and coping styles may be partly related to the task-oriented exercises with computerized visual feedback, which can be considered as a relevant tool to increase patients' motor output, involvement, and motivation during gait training [29]. Moreover, we have also argued that the effect of this advanced robotic gait training on cognition was due either to the improvement in attention/motivation (integrated biofeedback system monitors the patient's gait and provides real-time visual performance feedback to motivate the patient for active participation) or in mood, as depression may worsen cognitive impairment.

4. UPPER LIMB ROBOTIC REHABILITATION

Current upper-limb systems can be divided into two types: end-effector, including MIT/IMT-Manus, MIME, GENTLE/s, and exoskeleton, such as ARMin, Pneu-WREX, RUPERT, REHAROB) [30]. With end-effector systems, subjects hold a manipulandum that experiences robot-imposed forces. Therefore, all the forces and measurements are at a single interface that has the advantage of easy set-up for patients of different body sizes. An analysis of three studies that used different kinds of end-effector system (MIT-Manus, ARM Guide, MIME) did not show a significant difference with respect to ADL measures (i.e., FIM score), although comparisons at the level of impairment have not been made, nor between training in 2 versus 3 dimensions. With exoskeletal systems, the limb is enclosed in an actuated robotic suit conforming to the configuration of the limb. While a subject's limb can be constrained with an end-effector system to specify one limb configuration (e.g., ARM Guide), the mechanical flexibility of the exoskeletal type allows full specification of limb configuration and for forces to be applied and measured independently at each joint [30]. Exoskeletal systems have the advantage that forces can be applied and measured independently at each joint. To date, no studies have directly compared the two types of robotic system of system but differences can be expected based on motor control principles.

4.a. Armeo

The Armeo Therapy Concept improves the efficiency of therapy treatments because the exercises are self-initiated, self-directed, functional and intense. Interestingly, even severely impaired patients can practice independently, without the constant presence of a therapist, allowing patients to exploit their full potential for recovery [31].

The *ArmeoPower* has a robotic arm exoskeleton, with an electric lifting column for comfortable height adjustment, allowing active arm support in a large 3D workspace. It can be used for both the left and right arm and hand, and is adjustable to different arm sizes and to the height of the patient. A positioning system correctly aligns the shoulder joint for ergonomic actuation and the product promotes movement in all relevant joints, assessing torques and angles for shoulder, elbow, forearm, and wrist ROM.

The *ArmeoSpring* is specifically suited for patients who are beginning to regain active movement of the arm and hand, and has already proved to be successful in many clinics worldwide (Figure 3).

The Augmented Performance Feedback provided by the shared software platform, encourages and motivates patients to achieve a higher number of repetitions, and this leads to better, faster results and improved long-term outcomes.

The software also provides automatic, ongoing assessment of motor functions and patients can readily track their progress, helping them to grasp the initiative and reach towards recovery.

To date, only few studies have investigated the effect of such devices on stroke patients.



Figure 3. Post-stroke upper limb rehabilitation by using Armeo Power and Spring.

In particular, Colomer et al. [32] have found that Armeo® Spring is an effective tool for rehabilitating the affected arm in patients with hemiparesis secondary to ictus, even in the chronic stage.

5. FES-CYCLING AND BEYOND

Cycling exercise completed using a bicycle ergometer is usually used to supplement functional ambulatori training. The kinematic pattern of cycling exercise is very similar to that of walking; however, cycling exercise requires less balance capacity. Both walking and cycling exercise are cyclic and require the alternate muscle activation of antagonists of lower extremities in a well-coordinated manner. The intensity of cycling exercise can be controlled, and has been suggested to improve functional mobility, as well as balance, of stroke patients [33-34]. Other studies have reported that repetitive passive movement of spastic muscles can increase range of motion and reduce stiffness in the hypertonic joints of stroke patients [35-36]. The aforementioned studies also suggest that cyclic leg movement may provide a potential therapy for reducing hypertonia, one of the factors that prevent functional activity in stroke patients. The positive effect of cycling in hemiparetic patients is greatly potentiated by the use of Functional electrical stimulation (FES). In FES, computer technology sends low-level electrical impulses to specific muscles in patient's legs, arms, hands or other areas. The electrical stimulation can cause muscles to contract, which may promote increased muscle bulk or muscle control, and thus, may improve ROM, strength, and the functional use of hands, arms or legs. Previous studies have reported that functional tasks performed by hemiplegic hands show improvement after electrical stimulation treatment [37]. FES has also been used to improve walking ability in stroke subjects [36]. Moreover, the benefits of FES-

assisted cycling exercise include enhanced muscle strength and endurance, increased bone density, cardiopulmonary improvement, spasticità relief, and many other physiological and psychological advantages. Recently, similar FES-assisted cycling devices have been used for stroke patients, concluding that short term cycling training (with or without FES) is a useful therapeutic approach to reduce spasticity in stroke patients [35-36].

6. VITALSTIM

VitalStimtherapy is a special form of neuromuscular electrical stimulation (NMES) device, which has received FDA clearance to be used for treatment of pharyngeal dysphagia. It can only be administered under the direction of certified healthcare professionals (speech language pathologists and occupational therapists). VitalStim is a dual-channel electrotherapy system, which uses small calibrated electrical current delivered by specially designed electrodes to stimulate the muscles responsible for swallowing. At the same time, trained specialists help patients “reeducate” their muscles through rehabilitation therapy. A recent meta-analysis of the published literature has confirmed that no adverse events were identified and attributed a statistically significant positive treatment effect favoring the use of NMES on the throat in the rehabilitation of dysphagia [38].

Moreover, data by Li et al. [39] suggested that VitalStim therapy coupled with traditional swallowing therapy may be beneficial for post-stroke dysphagia. The mechanism by which Vitalstim may act in improving dysphagia could be related either to the repetitive and intensive swallowing exercises or to the electro-stimulation-induced of cortical plasticity.

7. COMPUTERIZED COGNITIVE REHABILITATION

Cognitive rehabilitation (CR) is a therapy to help brain-injured or otherwise cognitively impaired individuals to restore normal functioning, or to compensate for cognitive deficits [40].

The process of Cognitive Rehabilitation (CR) involves assessment of cognitive functions, identification of specific areas of impairment, goal setting and institution of appropriate rehabilitation techniques. Neuropsychological assessment is the first element of CR, necessary to establish the area of functional, emotional and behavior impairments and to serve as the basis for treatment planning. The computer-assisted cognitive rehabilitation (CCR) focus on similar neuropsychological processes but use computerized exercises that train attention and concentration, executive skills, perceptual motor skills and problem solving skills in game - like programs. CCR involves multimedia and informatics resources with the direct use of peculiar hardware systems and softwares, i.e., programs to reactivate neuropsychological performances compromised [41-42] (Figure 4).

Two types of CCR, i.e., the task-specific and the hierarchical approach, have typically employed different intervention approaches. In the specific approach, individuals are administered computer programs targeting specific cognitive deficits (for example, if the individual has attention problems, he/she will receive programs that reportedly train attention). In the hierarchical approach, individuals are trained on a sequence of programs that

are arranged hierarchically, training from fundamental to more complex cognitive functions (for example, the individual starts CCR with programs targeting attention, then moves up to visual spatial and perception, followed by memory retraining and, lastly, complex problem-solving programs) [43]. A recent Italian computerized cognitive tool, named ERICA, is used in the treatment of the cognitive dysfunctions following stroke. It includes a series of informatics exercises that are organized in five specific-domain (attention, memory, spatial cognition, verbal and non verbal executive functions), and finalized to stimulate the residual cognitive abilities post-stroke (Inzaghi, 2013). Another peculiar rehabilitative software, i.e., “Power-AFA”, is used to stimulate the language abilities (in production and comprehension) for the aphasic subjects.



Figure 4. A post-stroke patient CCR training at our Behavioral and Cognitive Laboratory.

As compared to conventional treatment, CCR has been demonstrated to be a promising methodology to optimize the rehabilitation outcomes following stroke [44].

8. VIRTUAL REALITY

Virtual Reality (VR) is a computer-simulated environment that can simulate physical presence in places in the real world or imagined worlds. Virtual reality can recreate sensory experiences, which include virtual taste, sight, smell, sound, touch, etc. Most current VR environments are primarily empirical experiences, displayed either on a computer screen or with special stereoscopic displays, and some regulated simulations include additional sensory information and emphasise real sound through speakers or headphones targeted towards witnesses [45]. Some advanced, haptic systems now include tactile information, generally known as force feedback in medical, gaming and military applications. The introduction of VR augmented sensorimotor rehabilitation was heralded as a therapy that promised ecologically valid, intensive task specific training. Additionally, it was suggested that VR

could deliver training intensity (repetitions and duration) associated with neuroplasticity and positive behavioral adaptations because it was particularly well suited to very high training doses [46].

The Virtual Reality Rehabilitation System (VRRS, by Khymeia Group) VRRS is based on previous research performed at the Department of Cognitive Science at MIT Massachusetts Institute of Technology in Boston. VRRS is conceived to put the patient in a situation to generate the augmented feedback towards his central nervous system (augmented feedback) through exercises performed in a virtual environment which help to develop knowledge of results of the movements (knowledge of results) and knowledge of the quality of the movements (knowledge of performance). A recent study has demonstrated that the reinforced feedback in virtual environment (RFVE) is more effective than traditional rehabilitation for the treatment of upper limb motor function after stroke, regardless of stroke etiology (i.e., ischemic, hemorrhagic) [47].

Moreover, VR exercise intervention for inpatient stroke rehabilitation has been found to improve mobility-related outcomes [48].

Following about 10 years of development and clinical trials, many prestigious international scientific publications have been released among which the Cochrane Review (the most authoritative international organization for evaluating clinical methods and medical devices) that has shown the fact that the virtual reality technology applied to neurological motor rehabilitation has a greater effect than using just the traditional rehabilitation [46].

9. TELEREHABILITATION

Telemedicine is defined by the American Telemedicine Association as the use of medical information exchanged from one site to another via electronic communications to improve patients' health status. Telerehabilitation (TR) is defined as rehabilitation specialists involved in applying computer-based technologies and telecommunications to improve access to rehabilitation services and to support independent living. This particular application of telemedicine exploits several aspects of rehabilitative medicine at a distance: tele-monitoring (patient assessment functioning and clinical management), teletherapy, tele-consultation, tele-mentoring and tele-education for professionals and caregivers [49].

Several National Health System guidelines recommend a reduction in the duration of patients' stays in hospital in order to minimize expenditure; with this in mind telemedicine could be utilized in facilitating early discharge support. A recent review of early discharge support post-stroke

illustrated that patients with mild to moderate disability were significantly less likely to be dead or dependent by the time of their scheduled follow-up, in comparison with those who received conventional care [50].

TR has been demonstrated to be a successful tool in improving the physical and cognitive outcomes of patients suffering from stroke. Through implementing the tele-cognitive rehabilitation activities, therapists can help patients to practice, and thus improve, their motor and cognitive skills [51]. Different studies have indicated a high possibility of accessing tele-rehabilitation services to enhance, in particular, memory, attention, problem-solving, and activities of daily living.

However, a recent systematic review has found insufficient evidence to reach conclusions about the effectiveness of telerehabilitation after stroke. Moreover, no randomised trials that included an evaluation of cost-effectiveness were found. Therefore, which intervention approaches are most appropriately adapted to TR remain unclear, as does the best way to utilise this approach.

10. CAREN

The (Computer Assisted Rehabilitation Environment) CAREN system, developed by MOTeK Medical (Amsterdam, Netherlands) consists of a motion capture system and a base driven by hydraulic and mechanical actuators. The CAREN system may also be equipped with varying degrees of virtual reality immersion ranging from a flat video, dual-channel audio, theater in its “base” model to a 360°, surround sound dome enclosure in its “high end” version. Real-time motion tracking technology enables the CAREN system to follow patient movements frame-by-frame for detailed kinematic and biomechanical analysis using up to 24 mounting locations. Numerous studies have also been conducted assessing over ground walking vs. virtual reality treadmill-based rehabilitation indicating that the CAREN system is an effective rehabilitation aid for patient assessment. The CAREN system is unique in that it allows the subject to be immersed in a realistic clinical environment, while therapist and physicians collect kinematic and kinetic data in order to plan future rehabilitation regimens.

The tool was first developed for the rehabilitations of wounded warriors [52].

Indeed, in everyday life, warfighters with lower extremity trauma may experience uneven terrain, cracks in pavements, slippery conditions, etc. – all potential scenarios that would increase fall risk or injury. However, when using the CAREN system, specific physical perturbations may simulate these environmental conditions in a more safe and controlled setting, and thus, new rehabilitation methods and gait/ prosthetic limb training may be developed for these individuals to mitigate falling risks outside of the clinic [53-54]. Although the applications for the CAREN system have continued to demonstrate how this rehabilitation tool is an important element in wounded warrior care, one distinct advantage of this system is that it provides physical and cognitive aid for those with multitrauma and traumatic brain injury, with emerging promising data also in stroke patients [55].

CONCLUSION

To ensure that after a stroke, people can return to a life that is meaningful to them and resume previous roles, rehabilitation needs to be either an intense or a lifelong process and should not stop at 3 months or at the end of formal rehabilitation programmes.

To provide ongoing rehabilitation in a sustainable way, a low-cost and effective delivery method needs to be found. Using assistive technology will improve patient's outcomes and reduce the cost of intervention.

Moreover, the use of telehealth systems will allow treatment to continue for longer, which may facilitate the transition from hospital to home and encourage people to direct their own rehabilitation in the longer term.

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Chapter 81

DIGITAL SUBTRACTION ANGIOGRAPHY IN VASCULAR IMAGING OF STROKE

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ABSTRACT

Stroke is the second most common leading cause of death in the world. Stroke could be classified into ischemic stroke, intracerebral hemorrhage and subarachnoid hemorrhage. Vascular imaging of stroke is crucial for early detection of primary and secondary causes of stroke. Digital subtraction angiography (DSA) has been regarded as the gold standard imaging method of choice in all patients with stroke. In this review, we have described the updated information of clinical and technical implementation of DSA in stroke imaging. The clinical utilities of DSA in imaging of carotid stenosis and implementation of contemporary DSA techniques in subarachnoid hemorrhage were also reviewed.

CAROTID STENOSIS

The measurement of internal carotid artery stenosis is one of the most important assessments made when symptomatic patients are considered for prophylactic internal carotid

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endarterectomy. Several different methods for measuring the degree of stenosis from carotid angiograms have been evolved, each holding varied definitions of what constitutes carotid stenosis. It is thus possible for the same angiogram to be reported as showing differing degrees of stenosis depending upon the particular method of measurement used. This is a potential source of confusion and frustration for both clinicians and researchers involved in this area. A uniform approach for the measurement of carotid stenosis is required, but a precise method to be adopted still remains to be declared.

Any method of measuring carotid artery stenosis, in this context, should first be shown to reliably predict the risk of ipsilateral ischemic stroke. The degree of ipsilateral carotid stenosis, as measured by the techniques used in the ECST[1] and NASCET, [2] has been clearly shown to predict stroke risk. More recently, the predictive powers of these methods and of the common carotid techniques have shown to be almost identical. Provided that there are no actual differences in the predictive power of these caliper techniques, the reproducibility of measurements by each technique becomes the significant issue. The aim of this study was to compare the reproducibility of these caliper techniques by assessing observer variability in reporting both intra-arterial DSAs and MRAs. The visual impression of stenosis, or “eyeballing” the degree of stenosis, remains a widely used technique by which angiograms are assessed in routine clinical practice.

In some recent in vitro studies, it has been illustrated that even though the accuracy rate of DSA is dependent upon the physical characters of the lesions, it exhibited more significant detection of severe stenotic lesions of about more than 70% over CTA and MRA between which no big difference was observed.[3] Despite, many utilities, accuracy of MRA still remains deficit for the surgical requisites determination in most cases of urgencies, further adjuring supplementary imaging analysis for confirmation.[4] Other advanced imaging technologies which could be augmented include digital ultrasonography(DUS), MRA with gadolinium contrast, and DSA. CEMRA(Contrast Enhanced Magnetic Resonance Angiography) could be an improved diagnostic in the determination of the severity of stenotic lesions and may even be much more efficient especially for the plaque morphology and is almost analogous to DSA and CTA.[5]

In another similar approach to describe the efficiency of different imaging techniques declared CTA (Computed Tomography Angiography) to be the most accurate method for the emergent diagnosis of carotid stenosis in contrast to MRA and DUS .Whereas showed an approximate analogy with blood-pool CEMRA.[6]

In a sixty-seven patients' set up, the approach of the techniques were substituted by their integrity and creditability to aid patient's interests and demands. It also emphasized upon alternate risks when intervened solely or in combinations as an alternative for DSA. With an accuracy rate of 80%, CTA, MRA and DUS showed close diagnostic alliance to DSA, independent of the surgical requisites but remained limited to the symptomatic carotid stenosis only. Thus, DSA still remains indispensable choice of investigation for carotid stenosis in a well equipped clinical set-up.[7-8]

Duplex ultrasound (DUS), magnetic resonance angiography (MRA) and computed tomography angiography (CTA) could also be beneficial for the follow up procedures succeeding a carotid endarterectomy in patients who have had an episode of stroke .While CTA and MRA aids a very clear 3D structures of the extra-cranial vessels, DSA-MRA privileges an extensive evaluation. These recent cost effective and non-invasive techniques

eventhough cant be relied upon completely but have successfully been able to gradually reduced the implementation of DSA pre-operatively under certain circumstances. [9-10]

MRA and CTA, both seem to share an approximate precision of $\geq 95\%$ in difference to DSA for assessment of carotid stenosis. [11] While the combination of DUS and CEMRA is beneficial for most of the typical cases of strokes, a consolidated data gained through DUS and CTA could be preferred for suggesting the need for surgical interventions with an extensive assessment.[12]

In case of advanced sub-arachnoid hemorrhage, an assisted DSA with Rotational Angiography (RA) surpasses the benefits of using it alone. Since 3D RA shows more specificity to aneurysms, offers a sophisticated pre-operative approach.[13] Another research revealed that the chances of errors could possibly be reduced by using any of these above mentioned techniques in a combination with DSA imaging but cannot be diminished completely.[14]

CEREBRAL ANEURYSMS

2D versus 3D DSA

2D DSA fails to expose the concealed vessels and their associated branches. On the other hand, 3D DSA commences a broader aspect of visualization for all super-imposed intracranial blood vessels. [15]

Certain parameters such as the size of the aneurysmal neck feeds as a significant base for the judgment of an aneurysm and for anticipating peri-operative procedures.[16] For this purpose, wide spread use of 2D and 3D DSA have been very helpful. Recent development of 3D DSA has lead to many comparative studies with previously existing methods of imaging including 2D DSA. Most researchers found 3D DSA to mimic 2D DSA images in many aspects but distinct provision of higher power of resolution.[17-18] 3D DSA is a comparatively rapid technology requiring a lower amount of radiation and contrast material.[19] 3D DSA magnifies the neck of an aneurysm more than 2D DSA, thus results in a mean difference of about 15% between both ratios.[19-20]Due to this fact, in spite of the presence of a highly advanced 3D DSA, 2D DSA remains popular than 3D DSA in most clinical settings due to the fact that it is more advantageous in many aspects such as quality, resolution, and limited need for further processing where 3D DSA remains deficient.[21]Risk of brain stem infarctions are examined via information from the study of the pontine arteries and their parallel circulations. Both 2D DSA and 3D-DSA are of equal importance for this approach of study. [22]

In a follow up set up, exploration of intracranial aneurysms succeeding an endovascular therapy was performed in which 3D DSA images outweighed that of 2D DSA. This study agreed with the use of 3D DSA over 2D DSA for predicting the rate of recurrences in high risk patients. [23]

3D DSA versus CTA

CTA is the gold standard choice for screening of acute onset of aneurysmal SAH. [24] But cannot be preferred as a primary choice for investigation of aneurysms preceding or following an endovascular therapy for an extensive assessment of anatomy of intra-cranial vasculature. [25] It is highly specific for the evaluation of severity of carotid stenosis. [2, 26] TA cannot provide enough evidences for minor bleeds. It is more likely to miss the detection of very small aneurysms sized <5mm. [27]

The exposure to radiation is roughly similar to DSA. CTA provides the maximum data without any threat of iatrogenic stroke since it doesn't include direct vascular interventions unlike DSA. On the contrary, DSA surpasses CTA as it helps to observe arteriovascular malformations (AVMs) and fistulas at controlled time intervals. [28]

3D DSA versus MRA

Blood-pool-enhanced MR angiography, is one the latest advanced MRA method which could also be implemented for a detailed assessment of the severity of carotid stenosis. [29] 4D DSA, briefly conferred as a 3D DSA process at regular intervals following the contrast flow pattern; proved to be a redeemed mode of imaging which is programmed favorably for an extensive access to the AVMs and remains unbiased upon the direction and thus, independent of all the intervening intracranial structures. It has been able to surpass all other previous conventional methods for this purpose. Further, the obtained data from a 4D DSA helps revealing all concealed information relevant to a stroke. This study needs to be emphasized upon in future for its other benefits. [30]

COLLATERAL CIRCULATION

DSA is the gold standard method of choice for imaging of collateral circulation in patients with stroke. Cerebral collateral circulations can be categorised into primary and secondary collaterals. Primary collaterals refer to the components of the circle of Willis and the ophthalmic artery and leptomeningeal blood flow constitute secondary collateral vessels. [31,32] Leptomeningeal collateral is associated with infarct growth and outcome in patients with acute ischemic stroke. [33] Good leptomeningeal collateral is reported to predict better outcome after acute ischemic stroke, even adjustment for known prognostic factors. [34] The underlying mechanism might be that good collateral flow maintain the ischemic penumbra before reperfusion.

Although several imaging method have been utilized in evaluation of collateral flow in patients with stroke, DSA remains the gold standard imaging method of choice. DSA can visualize the extent and number of collateral flow. TIMI Grade Flow is the most commonly used scoring system for evaluation of collaterals in patients with acute myocardial infarction and stroke. [35] It is originally derived from patients with cardiovascular disease. Now, the scoring system is widely used for assessment cerebral collateral flow. The TIMI grade ranges from 0-3 referring to levels of blood flow assessed during DSA. TIMI Grade 0 flow indicates

the absence of any antegrade flow beyond an occlusion. TIMI 1 flow is penetration without perfusion. The antegrade flow beyond the occlusion with incomplete filling of the distal vascular bed. TIMI 2 flow represents slow antegrade flow which completely reperfuse the distal artery. TIMI 3 is normal flow that lead to complete filling of the distal vascular territory.

Recently, CT angiography have been increasingly used in assessment of collateral flow.[35] Several leptomeningeal collateral scoring systems have been proposed and predict functional outcome. CTA source images were usually used to assess for the presence of collateral flow. The region of the Sylvian sulcus arteries were compared with the contralateral side. The grading system was based on the absence or presence of the vessels. However, considerable regional variability in the presence of these arteries were not considered in most scoring systems. CTA based collateral grading system has demonstrated good interobserver reliability and correlated with clinical outcome. The recent development of dynamic CTA imaging with perfusion imaging has greatly advanced the role of CTA in assessment of collateral circulation. MR angiography is less commonly used than CTA and DSA for assessment of collateral flows. 3D TOF or contrast-enhanced MRA are commonly used for visualization of collaterals. However, the signal intensity in is variable due to different acquisition methods.[37,38] TCD also provide noninvasive measurements of collaterals, but are less accurate than MRA. TCD is primarily used for evaluation of circle of Willis component vessels. The color-coded duplex ultrasonography may be improved with contrast administration.

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Chapter 82

MOYAMOYA: A FOCAL ICA PATHOLOGY CAUSING ISCHEMIC AND HEMORRHAGIC STROKE

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ABSTRACT

Moyamoya is a cerebrovascular condition defined by the chronic progressive stenosis of the intracranial internal carotid arteries (ICAs) and their major branches. This stenosis predisposes patients to ischemic and hemorrhagic stroke resulting in focal disabilities and progressive cognitive impairment. *Moyamoya disease* predominately occurs in Asian children, and is now thought to result from a particular genetic mutation. This does not, however, explain the occurrence of moyamoya vasculopathy in many adults throughout the world. *Moyamoya syndrome* may occur in patients with other well characterized auto-immune diseases or coagulopathies. While the etiology of moyamoya remains unknown, there is an increasing body of literature that suggests inflammation contributes to its pathogenesis and/or progression. Studies have demonstrated smooth muscle cell proliferation and infiltrating T cells and macrophages in blood vessel walls, while other studies demonstrate elevated levels of endothelial adhesion molecules and cytokines in the cerebrospinal fluid and plasma of moyamoya patients. However, to more readily study the development and pathogenesis of moyamoya, and to create potential therapies for this condition, experimental animal models are needed. Here, we will review the transgenic mouse model engineered to study the genetic contribution to the disease, as well as describe the recently-developed surgical model to study moyamoya syndrome. Animal models can undergo similar radiologic imaging that is currently used to diagnose moyamoya and can be used to study the effects of inflammation on blood vessel function. Importantly, animal models can also be used to study the influence of moyamoya on cognitive impairment, an increasingly recognized chronic effect of the pathology. While moyamoya is a rare condition, the natural history can have a

devastating prognosis, and current treatment options are limited. Improving our knowledge about this condition will help in developing novel therapeutic approaches, and may help in understanding the role of neuroinflammation on carotid artery pathologies.

INTRODUCTION

The arterial blood supply of the brain and much of the spinal cord is derived from the internal carotid and vertebral arteries [1]. The internal carotid artery (ICA) ascends through each side of the neck and at the base of the brain gives rise to the ophthalmic artery, anterior choroidal artery, and the posterior communicating artery. It then bifurcates into the middle cerebral artery (MCA) and anterior cerebral artery (ACA), which supply blood to large portions of the frontal, parietal, and temporal lobes [1]. The ICA is divided into seven segments, as classified by Bouthillier et al., according to the anatomy surrounding the ICA and the compartments through which it travels; cervical, petrous, lacerum, cavernous, clinoid, ophthalmic, and communicating [2].

Moyamoya is a chronic cerebrovascular occlusive condition that predisposes affected patients to stroke in association with progressive stenosis of the supraclinoid segment of the ICAs and their proximal branches [3-5]. Reduced blood flow in these major arteries leads to ischemia, while the compensatory development of new blood vessels at the base of the brain leads to hemorrhage [3, 6]. The characteristic appearance of these abnormal neovascularizations was described on angiography as a “vague or hazy puff of smoke”, which is *moyamoya* in Japanese [7]. Extensive investigations of patients with this characteristic angiographic finding have been conducted over the past 50 years. Suzuki et al. divided the progression of moyamoya cerebrovascular pathology into six stages, including narrowing of the ICA and initiation of collaterals (stages 1-2), significant narrowing of the ICA and intensification of collaterals (stages 3-4), and complete blockage of blood flow through the ICA with concomitant disappearance of the collaterals with reliance on the external carotid branches to supply blood to the brain (stages 5-6) [7, 8]. During these various stages, patients suffer from headache, ischemic stroke, transient ischemic attacks (TIAs), seizures, and hemorrhagic stroke [4], which lead to focal disabilities and progressive cognitive impairment [9, 10].

Moyamoya disease (MMD) etiology remains unknown. However, there is a familial tendency in which individuals with a first-degree relative with MMD have an increased risk of developing the disease [11-13]. There is also a predilection for people of Japanese and Korean ancestry as disease incidence in East Asia is approximately 10 times higher than in Western countries [14]. Indeed, genetic studies have demonstrated possible linkage between markers located on several different chromosomes and familial occurrence [15-18]. In addition, studies identified the ring finger protein 213 (RNF213) gene as a susceptibility gene for MMD [19, 20]. Although hereditary factors may be involved in susceptibility to MMD, there are a growing number of cases that are sporadic. Moyamoya-like vasculopathies are now observed throughout the world in people of various ethnic backgrounds, including American and European populations [21-23]. These patients exhibit angiographic features of moyamoya vasculopathy along with other well-characterized system disorders; they are considered to have moyamoya syndrome (MMS). MMS-associated co-morbidities include hypertension, obesity, hyperlipidemia, thyroid disease, sickle cell disease, neurofibromatosis

type 1, Down syndrome, diabetes, and a variety of other auto-immune diseases [4, 12, 21, 23]. While an extensive number of reports exist to describe MMD, less is known about MMS. In fact, only recently have reports in literature begun to distinguish between the two. MMD incidence in the Japanese is calculated to be 0.35 per 100,000 population with a female-to-male ratio of approximately 1.8:1 [24]. In the United States (Washington and California), combined MMD and MMS incidence is reported to be 0.086 per 100,000 [25]. In Kentucky, however, a MMS incidence of 0.7 per 100,000 is reported [23], indicating regional differences. Moyamoya has a peak occurrence at two ages (children and adults), each with distinctive clinical presentation. Nearly half of MMD patients are diagnosed prior to 10 years of age and predominantly suffer from transient ischemic attacks, ischemic strokes, cognitive decline, epilepsy, and involuntary movements [8]. In contrast, adults diagnosed in their 20's – 40's commonly present with intracranial hemorrhage; this is the most common age group for MMS patients [5, 8]. While bilateral stenosis/occlusion of the ICA is most common in MMD, unilateral stenosis/occlusion can occur, especially in MMS patients [4].

The histopathology of moyamoya has been of interest for a number of years. Examination of the ICA and its major branches found a fibrocellular intimal thickening with proliferation of vascular smooth muscle cells [26-29]. Fibrin deposits, fragmented elastic lamina, and microaneurysms have also been observed [4, 5, 30]. These vessel pathologies are thought to be responsible for the stenosis and resultant ischemic and hemorrhagic stroke in these patients. In addition, the immune system may be a key factor in the pathogenesis and/or progression of moyamoya. Histopathological studies have shown macrophages and T cells infiltrate the thickened intima of occlusive vessels [27, 31]. Furthermore, immunomodulatory molecules such as soluble endothelial adhesion molecules and cytokines, as well as chemokines and growth factors are elevated in the cerebrospinal fluid (CSF) and serum of MMD patients, respectively [32-34]. The lack of animal models makes it difficult to ascertain the degree of involvement of inflammation and co-morbid conditions on the mechanisms of pathogenesis. In this chapter, we will summarize alterations in immunity in moyamoya (MMD and/or MMS) patients and how inflammation may contribute to moyamoya pathology. We will also discuss the advancement of animal models and how they may aid in the development of novel treatments or therapies.

INFLAMMATION

Inflammation is known to be an important factor in a wide variety of diseases. In the continued efforts to discover the etiology of moyamoya, the role of the immune system is gaining attention. However, it remains a great debate as to whether inflammation is a causal factor in moyamoya or if it is a result of the stenosis and ischemia associated with the condition. It is important to note that many previous investigations do not distinguish between MMD and MMS. Therefore, some cases as reported below to occur in MMD patients may, in fact, occur in MMS patients as well. We will review the immunological alterations that are observed in patients with moyamoya (Table 1) and its potential participation in the development and/or progression of disease.

Table 1. Immunological Changes in Moyamoya Patients

	Changes in MMD	Role in MMS	General role in inflammation	References
Leukocytes				
Macrophages	Increased in vessel walls; no change in the intima and tunica	Increased T cells in HIV-1 infected patients with MMS	Phagocytosis; Antigen presentation; Cytokine and growth factor	27; 35; 36
T cells	Increased in vessel walls, blood		Cytokine and chemokine secretion; Stimulation of antibody production; Recruitment of other immune cells	27; 31; 40
B cells	No change in vessel walls		Antibody production; Cytokine secretion; Antigen presentation	27
Cytokines				
TGF-β	Increased in blood, SMCs; No change in CSF		Anti-inflammatory	31; 41; 42
IL-10	Increased in blood		Anti-inflammatory	31
TNF-α	Increased in blood; no change in blood		Pro-inflammatory	31; 33
IL-17	Increased in blood		Pro-inflammatory	31
IL-6	Increased in blood; No change in blood		Pro-inflammatory	31; 33
IL-23	Increased in blood		Pro-inflammatory	31
IL-1b	Increased in blood		Pro-inflammatory	33
IL-2	No change in blood		Pro-inflammatory	31
IL-4	No change in blood		Pro-inflammatory	31
INF-γ	No change in blood		Pro-inflammatory	31
IL-8	No change in CSF		Pro-inflammatory	33
Chemokines and adhesion molecules				
ICAM-1	Increased in blood; Increased in CSF	Increased in CSF	Stabilizes cell-to-cell interaction; promotes leukocyte migration	31; 34
VCAM-1	Increased in blood; Increased in CSF	Increased in CSF	Promotes adhesion of leukocytes to vessels; promotes leukocyte	31; 34
E-selectin	Increased in CSF	Increased in CSF	Recruits leukocytes to the site of injury; mediates leukocyte "rolling"	34
CXCL5	Increased in blood		Increases chemotaxis of angiogenic neutrophils	49
CXCL12/ SDF-1α	Increased in blood		Activates and attracts lymphocytes; recruits EPCs from the bone marrow	50
CCL2/ MCP-1	Increased in blood		Recruits leukocytes to site of	33
Growth Factors				
VEGF	Increased in dura, glial cells, blood; decreased receptors; no change in CSF	Increased in the CSF	Promotes angiogenesis and vasculogenesis	33; 44; 52
bFGF	Increased in CSF, SMCs and ECs in STA, meningeal and vascular		Many mitogenic and cell survival functions	54; 55; 56
HGF	Increased with receptor in tunica media and intima, blood, CSF		Regulates cell growth, cell motility, and morphogenesis	44; 56
PDGF	Increased in blood		Regulates cell growth and division; in particular angiogenesis	33
G-CSF	Increased in blood		Stimulates hematopoietic cells from bone marrow into blood; promotes neutrophil survival, proliferation	
Other Factors				
sCD163	Increased in the blood		Soluble marker of cells of a macrophage lineage	32
Autoantibodies	Increased in the blood, CSF	Increased in the blood	Antibodies that target and react to a person's own tissue	62; 63; 64; 66
MMD, moyamoya disease; MMS, moyamoya syndrome; CoW, Circle of Willis; SMC, smooth muscle cell; TGF, transforming growth factor; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; ICAM, intercellular cell adhesion molecule; CSF, cerebrospinal fluid; VCAM, vascular cell adhesion molecule; EPC, endothelial progenitor cell; EC, endothelial cell; STA, superficial temporal artery; GF, growth factor; VEGF, vascular endothelial GF; bFGF, basic fibroblast GF; HGF, hepatocyte GF; PDGF, platelet-derived GF; G-CSF, granulocyte colony stimulating factor				

MMD, moyamoya disease; MMS, moyamoya syndrome; CoW, Circle of Willis; SMC, smooth muscle cell; TGF, transforming growth factor; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; ICAM, intercellular cell adhesion molecule; CSF, cerebrospinal fluid; VCAM, vascular cell adhesion molecule; EPC, endothelial progenitor cell; EC, endothelial cell; STA, superficial temporal artery; GF, growth factor; VEGF, vascular endothelial GF; bFGF, basic fibroblast GF; HGF, hepatocyte GF; PDGF, platelet-derived GF; G-CSF, granulocyte colony stimulating factor

Leukocytes

Macrophages

Macrophages are an important component of the innate immune system and are responsible for phagocytosis of debris, dead cells, and foreign invaders. Their production of

reactive oxygen species, presentation of antigens to T cells, and secretion of soluble factors such as cytokines and growth factors can induce a diverse immune response [35]. It is thought that macrophages may be particularly relevant to MMD pathophysiology because of their role in another vascular disease, atherosclerosis, which is characterized by the deposition of lipids and resultant narrowing of arteries [27]. In this disease, macrophages penetrate the vasculature where they have both protective and pathogenic roles [35]. While it is unknown whether these factors hold true in MMD, there is conflicting evidence to suggest that macrophages are associated with the pathology.

One immunohistological study of the Circle of Willis isolated from 2 MMD patients post-mortem found very few CD68+ monocytes (i.e., macrophages) in the intima and tunica media [36]. However, Masuda et al. observed macrophages (subset not identified) that infiltrated the vessel walls of 6 MMD patients [27]. Interestingly, high concentrations of soluble CD163, a marker of activated M2 macrophages, has been measured in the blood of MMD patients [32, 37]. M2 macrophages exhibit diverse roles in vascular disease, including atherosclerosis, and are thought to be protective because of their anti-inflammatory nature and role in tissue repair [35]. However, one study found that CD163+ M2 macrophages from human atherosclerotic lesions correlated with pathological thickening of the intima and related stenosis, key features of MMD [38].

Given that MMD patients suffer from aberrant angiogenesis (excessive formation of collateral vessels) and increased vascular permeability, it is interesting to note that CD163+ M2 macrophages are also involved in intra-plaque angiogenesis and vascular permeability in atherosclerosis [38]. From these studies, we can conclude that macrophages have a complex role in the pathophysiology of MMD that cannot be labeled as exclusively protective or pathological. Additionally, increased levels of soluble CD163 in the blood of patients with MMD as well as autoimmune and inflammatory disease, indicates its potential to serve as a biomarker for pathogenic alterations to immunity during moyamoya [32].

T Cells

T cells are a type of white blood cell that are important for the adaptive immune response. Like macrophages, T cells are recruited to the blood vessel wall in vascular disease and have been found to infiltrate vasculature in MMD patients [27]. They respond to antigen presentation by macrophages and subsequently differentiate into various subtypes, such as effector, helper, or memory T cells [39, 40]. CD4+ T cells (i.e., helper T cells) play a critical role in immune protection and pathology by stimulating antibody production by B cells, recruiting other immune cells to the site of inflammation, and secretion of cytokines and chemokines [41]. Interestingly, significantly increased levels of CD4+ T cells have been found in the blood of MMD patients [31]. Given the diverse roles of CD4+ T cells in the immune system, the CD4+ populations were further divided into two subsets for analysis: Th17 cells and regulatory T cells (Tregs). Th17 cells are a pathogenic effector subset that contribute to inflammatory and autoimmune disease. The role of Th17 cells in vascular disease is controversial. In mouse models of atherosclerosis, blocking interleukin-17 (IL-17), the primary cytokine produced by Th17 cells, can either attenuate or accelerate atherosclerosis depending on the model used [40]. Circulating Th17 cells were shown to be increased in MMD patients, indicating the occurrence of systemic inflammation [31]. MMD patients also had increased levels of Tregs [31], an anti-inflammatory subset of T cells that counteract the immune response by preventing T cell proliferation, producing

immunosuppressive cytokines and counteracting autoimmunity [40]. While an increase in both pro- and anti-inflammatory T cell subsets seem contradictory, further analysis of the isolated Tregs showed they had a diminished suppressive function. In fact, the subset of Tregs identified in the MMD patients, known as FR_{III} (CD4⁺CD25⁺FoxP3^{low}CD45RA⁻), have an “unstable” phenotype and can transform into pro-inflammatory Th17 cells [40]. In addition, higher levels of Tregs were associated with more severe MMD, however no association between Th17 cells and disease severity was observed [40]. Elevated populations of T cells in the blood may be indicative of systemic inflammation in MMD patients and infiltration into the blood vessels suggest a role for T cells in moyamoya pathology.

Cytokines, Chemokines and Growth Factors

Cytokines

Cytokines are a broad class of small proteins that are important for cell signaling. They are produced by a wide range of cells, including immune cells and endothelial cells, and are important for the modulation of the immune system. Cytokines can be further broken down based on their function, cell of secretion, or target of action.

Cytokine concentrations in the blood and CSF of MMD patients also yield conflicting results. Transforming growth factor-beta (TGF- β), an anti-inflammatory cytokine produced by Tregs, was increased in the serum by three-fold compared to controls [41]. In addition, cultured smooth muscle cells (SMCs) derived from the superficial temporal arteries of MMD patients have increased expression levels of TGF- β , relative to healthy controls and patients with atherosclerosis [41]. However, TGF- β levels are not increased in the CSF of MMD patients [42], possibly suggesting a more systemic, rather than central, anti-inflammatory response for TGF- β . Additionally, IL-10 serum levels were also increased in MMD patients [31]. Its role in vascular disease is thought to involve the prevention of leukocyte-endothelial cell interactions and the inhibition of cytokine and chemokine production by other lymphocytes [43].

To further complicate our understanding of the role of cytokines in MMD, serum levels of certain pro-inflammatory cytokines (IL-17, IL-23, IL-1 β) are shown to be increased, while other pro-inflammatory cytokines (IL-2, IL-4, interferon (IFN)- γ) were not different from control patients [31, 33, 44]. Tumor necrosis factor-alpha (TNF- α) is elevated in the serum of MMD patients, however other investigators found no change [31, 33]. Finally, arterial SMCs treated with IL-1 β stimulated cell migration and DNA synthesis when the cells were derived from control patients, but had an inhibitory effect on cell migration and growth of the MMD patient cultures [45]. Taken together, this data suggests a complex interplay between pro- and anti-inflammatory cytokines in MMD patients that could be clarified by mechanistic neuroimmune studies in animal models of moyamoya.

Chemokines and Adhesion Molecules

A subset of cytokines, known as chemokines, have attracted interest from MMD researchers because they promote the migration and adhesion of leukocytes to vessel walls, as well as the diapedesis of immune cells into surrounding tissue, making them important during vascular disease [46]. Adhesion molecules, as the name implies, promote the adhesion of

leukocytes to vessel walls and, in conjunction with chemokines, promote the recruitment of leukocytes [47]. Chemokines and adhesion molecules are critical for the migration of leukocytes to vascular tissue and their role can be characterized in 4 major steps: (1) selectin-dependent tethering and rolling of the immune cell along the vessel wall, (2) adhesion via chemokines and their receptors, (3) integrin-dependent firm adhesion, and (4) egress across the endothelium [47]. MMD patients present with increased serum expression of intracellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [31]. ICAM-1, VCAM-1, and E-selectin are also increased in the CSF of MMD patients, providing support for the importance of adhesion molecules in both the periphery and CNS [34]. In murine models of vascular disease, treatment with VCAM-1 antibodies or VCAM-1 deficiency have been shown to be protective against atherosclerosis [47]. Genetic deletion of selectins in mouse models of vascular disease also induce protection, albeit to a lesser extent [47].

Changes in chemokine levels have been linked to MMD *in vivo* and *in vitro*. Studies report that while chemokine levels were not altered in the CSF of MMD patients [33], three chemokines were elevated in the blood [32, 48]. First, patients exhibit higher serum levels of CXCL5, a chemokine produced by both immune and vascular endothelial cells in response to pro-inflammatory stimuli [49]. CXCL5 promotes angiogenesis and neutrophil chemotaxis, both of which could contribute to MMD pathology, and has been linked to autoimmune diseases [50]. Second, patients exhibit higher plasma levels of CXCL12, a powerful chemotactic for lymphocytes. It modulates angiogenesis by promoting the trafficking of endothelial progenitor cells from the bone marrow to the peripheral blood [48]. Third, MMD patients present with increased plasma levels of CCL2 [33]. CCL2 deficiency or the genetic deletion of its receptor, CCR2, is protective in murine models of atherosclerosis, reducing lesion size and macrophage infiltration [46]. Additionally, *in vitro* studies found that co-cultures of endothelial colony-forming cells (ECFCs) and smooth muscle progenitor cells (SPCs) show a CCL5-dependent movement of SPCs [51]. SPC migration is increased in co-cultures from MMD patients, indicating chemokines may be consistently upregulated in MMD, further exacerbating vascular inflammation [51].

Growth Factors

Various growth factors have been shown to be increased in the CSF and blood of MMD patients, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), and granulocyte colony-stimulating factor (G-CSF). In fact, VEGF may play a central role in MMD pathology, as it stimulates pathological vasculogenesis, increases vascular permeability in intracranial lesions, and enhances angiogenesis during cerebral ischemia [28]. VEGF expression is also increased four-fold in the dura of adolescent MMD patients and was identified in glial cells of an adult patient with MMD [28, 52]. Increased VEGF expression correlates with higher levels of the aforementioned anti-inflammatory cytokine, TGF- β , as well as higher levels of matrix metalloproteinase 9 (MMP-9), an enzyme that degrades the extracellular matrix [31, 33]. The breakdown of the extracellular matrix can lead to vascular instability and increase the likelihood of hemorrhages. However, despite the higher levels of VEGF, soluble VEGF receptors are lower in MMD patients compared to healthy controls [53].

Another critical growth factor for the development of moyamoya may be bFGF, which enhances vascular smooth muscle growth [28]. Immunohistochemical examinations of surgical specimens obtained from MMD patients found that bFGF is overexpressed in multiple tissues, including the superficial temporal artery (STA), endothelial cells, SMCs, dura mater, and vessels of the Circle of Willis [36, 54]. High levels of bFGF may lead to vessel stenosis given that bFGF has also been found in abnormally thickened tunica media [42]. Additionally, two studies demonstrate elevated concentrations of bFGF in the CSF of MMD patients compared to atherosclerotic patients and healthy controls [55, 56].

Several other growth factors are also linked to MMD, including HGF, G-CSF, and PDGF. HGF stimulates proliferation of endothelial cells and migration of cultured arterial SMCs derived from MMD patients, making this yet another factor that could contribute to pathological angiogenesis [28, 57]. Nanba et al. determined that expression of HGF and its receptor c-Met are increased in the tunica media and intima of vessels from MMD patients [58]. Additionally, there is a higher concentration of CSF-derived HGF in MMD relative to patients with cervical spondylosis and internal carotid occlusions [44, 58]. G-CSF mobilizes hematopoietic stem cells in the bone marrow and promotes neutrophil survival, proliferation and differentiation. It can also promote angiogenesis, further linking it to MMD pathology. Serum levels of G-CSF were found to be 1.5-fold higher in children with MMD compared to healthy controls [59]. Finally, higher concentrations of PDGF in the plasma of MMD patients have been reported [33]. *In vitro* data indicate a complex relationship between SMCs and PDGF. SMCs derived from the superficial temporal arteries have a blunted response to PDGF, however, PDGF-receptors are downregulated in arterial SMCs isolated from MMD patients [60, 61]. Despite this, it appears PDGF still exerts a differential effect on MMD SMCs, as it stimulates cell migration to a greater extent than in control cultures [57].

Autoimmunity

Autoantibodies

MMD patients present with higher levels of circulating autoantibodies in the blood and CSF [62, 63]. Sigdel et al. found 165 autoantibodies with a greater than 2-fold change relative to control subjects. Some key autoantibodies they discovered in serum were the B-cell antigen receptor complex associated protein alpha chain (CD79A), ephrin-A3, which is critical for integrin-mediated T lymphocyte interactions, and CD7, a surface marker for cytotoxic CD8+ T cells [63]. Molecular pathway analysis showed that reactive antigens were involved in the following pathways: neurological disease, tissue development, cellular growth and proliferation, and muscular system development [63]. MMD patients had a higher antigenic response to proteins in the immunological and inflammatory response, including interferon and the tumor necrosis factor superfamily [63]. Other autoantibodies detected in MMD patients include a natural T cell toxic autoantibody, in the serum of 5 out of 23 patients [64], thyroid autoantibodies associated with “aggressive presentation” (i.e., hemorrhage, major stroke, transient ischemic attacks), and anti-thyroperoxidase antibodies that increased the likelihood of ischemic stroke in MMD patients [65, 66]. MMD autoantibody profiles of individuals with active disease compared to those that had received revascularization surgery found that post-operative patients had lower expression of autoantibodies compared to

individuals with active disease [63], suggesting that the vascular pathology induces an autoimmune response that can be countered by treatment.

Autoimmune Disorders and Other Predisposing Diseases

As mentioned in the introduction, moyamoya is associated with several predisposing diseases related to the immune system. There is a higher prevalence of autoimmune disorders, particularly type 1 diabetes and thyroid disease, in midwestern US populations [21, 66]. Similarly, in an epidemiological study of a Western Chinese population, there was a greater likelihood of type 1 diabetes and Graves disease in MMD patients relative to the general Chinese population [67]. In fact, the overall prevalence of autoimmune disease in MMD was up to 31%. Juvenile-onset autoimmune disease is also associated with MMD in both pediatric and adult patients [68]. Prior infections and chronic inflammation are also risk factors for MMD. Serum levels of *Propionibacterium acnes* (*P. acnes*) antibodies were higher in individuals with MMD compared to healthy controls [69]. When rats were infected with *P. acnes*, they developed MMD-like changes to their internal carotid arteries. Epidemiological studies show that many MMD patients have a history of inflammation above the neck, in particular, recurrent chronic tonsillitis [70]. Taken together, this suggests that prior inflammatory or autoimmune disorders could be predisposing factors that increase the risk for developing moyamoya, and specifically MMS.

Genetics

HLA

There have been many efforts to identify genetic risk factors that could predispose individuals to developing MMD. Many of the relevant genes identified have been linked to immune function. Several studies performed genotyping of type I and II human leukocyte antigens (HLA) in patients with MMD [71]. Molecules encoded in the HLA locus play many roles in the immune system: antigen presentation, inflammation regulation, the complement system, and innate and adaptive immune cell responses. HLA molecules are also critical for self/non-self-recognition and HLA polymorphisms are closely linked with many autoimmune disorders [72]. MMD studies found associations between HLA alleles and MMD in both Asian and Caucasian European populations [71]. While many alleles have been identified, the strongest candidate is HLA-DRB1*13, which was significantly associated with MMD in both Korean and European populations, with a higher frequency of this phenotype in MMD patients [73, 74]. Interestingly, the HLA-DRB1*13 allele is believed to be protective against other autoimmune disorders, as this allele is underrepresented in patients with systemic lupus erythematosus, psoriasis, rheumatoid arthritis, systemic sclerosis, multiple sclerosis, and myasthenia gravis [75]. However, patients with systemic lupus erythematosus possessing the HLA-DRB1*13 allele were at greater risk for vascular events, and this allele increases susceptibility to ischemic stroke, indicating that this allele may have a dual pathogenic and protective role [76, 77].

Ring Finger Protein 213 (RNF213)

In addition to HLA, there has been significant interest in ring finger protein 213 (RNF213) as a single nucleotide variation in the RNF213 gene is strongly associated with MMD onset in both familial and sporadic cases [78]. Mutational analysis of RNF213 in 72 Japanese MMD patients found that 95% of familial MMD cases and 73% of sporadic MMD cases exhibited a founder mutation, p.R4859K, in the gene [19]. Gene ontology analysis demonstrated significant enrichment in the following categories: immune response, response to virus, defense response, inflammatory response, and innate immune response [78]. RNF213 is expressed in high levels in mature lymphocytes, further linking this gene to immune function [79]. To date, only one small study of two European Caucasian siblings with MMS has investigated the relationship between genetic mutations and MMS. This study did not find any association between the RNF213 gene or HLA alleles and MMS. However, it is difficult to assess the validity of these findings given the small sample size.

Animal studies are showing a clear link between RNF213 and the regulation of vasculature. RNF213 is critical to vascular development as *rnf213* knockdown in zebrafish results in irregular formation and abnormal sprouting of craniocervical vessels [20]. However, this finding has not been recapitulated in mice. RNF213 knockout, transgenic, and knock-in mice under basal conditions do not exhibit any changes in cerebrovasculature [80]. Under stressful conditions however, such as hypoxia, a common complication in MMD patients, transgenic mice expressing an orthologue of human RNF213 exhibit impaired compensatory angiogenesis in their cerebral cortex [81], as experienced by MMD patients. Dysfunctional changes in vasculature have been further linked to immunity through *in vitro* studies of RNF213. Induced pluripotent stem cells derived from the vascular endothelial cells of MMD patients with one or two RNF213 risk alleles have lower angiogenic activity compared to unaffected fibroblast donors. In addition, interferon (IFN)- β , an anti-angiogenic cytokine, suppresses angiogenic activity in endothelial cells via the induction of RNF213 [81]. In contrast to these studies, siRNA-mediated knockdown of RNF213 decreases the angiogenic response of endothelial cells, via upregulation of MMP expression, which has deleterious effects on the basement membrane surrounding endothelial cells [78]. Overall, the presence of RNF213 appears to lead to decreased angiogenesis, though conflicting reports exist. Decreased angiogenesis may account for moyamoya effects in the cortex, but does not account for the increase in neovascularizations observed at the base of the brain. It's clear there is a complex relationship between RNF213, inflammation, and angiogenesis in MMD pathology that requires further investigation.

Growth Factors and Cytokines

Several other genes related to cytokines and growth factors have also been associated with MMD. DNA analysis of 40 European MMD patients found two single nucleotide polymorphisms (SNPs) that may be possible genetic risk factors for developing MMD [82]. There was a significant association between a SNP in the promoter region of the PDGF β gene and in the first exon of TGF β 1, which encode a growth factor and a cytokine with immunological relevance, respectively. However, a study by Liu et al. of Japanese and European MMD patients found no association between the TGF β 1 gene and MMD, thus calling into question whether TGF β 1 is truly a susceptibility gene for MMD [83]. Linkage analysis also shows an association between chromosome 3p and MMD. Importantly, chromosome 3p encodes proteins involved in multiple immunological signaling cascades,

including IL-5 receptor alpha (IL5RA) and TGF- β receptor II (TGF β R2) [84]. The VEGF-634G allele has also been linked to pediatric MMD and poor collateral vessel formation after surgery, but no studies to date have replicated this finding [85].

TREATMENT

Unfortunately, there are no therapies available to prevent or reverse the progression of ICA stenosis. Instead, the focus is on reducing the risk of repeated strokes, hemorrhages, or general hypoperfusion of the brain. Antiplatelet treatment, such as aspirin, is commonly utilized for the prevention of stroke. However, evidence for the efficacy of antiplatelet treatment in moyamoya is lacking. In a recent study of MMD patients in Japan, antiplatelet therapy could not prevent recurrent ischemic infarction, possibly due to the fact that the nature of the ischemia is mainly hemodynamic, not embolic [86]. The pathologic changes of the ICA and associated vessels are not prone to platelet adhesion, and therefore antiplatelet therapy may not be effective for preventing ischemic stroke [87]. Surgical intervention to augment intracranial blood flow is currently the only other treatment option, and does improve long-term outcome in patients [87]. Direct bypass involves connecting the superficial temporal artery (STA) from the scalp to a cerebral artery, most commonly the middle cerebral artery (MCA). While improvement in blood flow is observed immediately after surgery, the procedure is technically difficult as the arteries have often shrunk and the vessel walls are fragile [87, 88]. Indirect bypass techniques rely on spontaneous angiogenesis between the cortical surface and donor tissues. The procedure is relatively easy to perform and widely used, though beneficial effects are not immediate and may take several months to develop [89-91].

There are several commonly used indirect bypass procedures: Encephalo-duro-arterio-synangiosis (EDAS) involves making a burr hole in the skull directly below the STA where it is sutured to the dura [92, 93]. This induces the formation of small arterial vessels to the brain without disrupting the integrity of the STA. Encephalo-myo-synangiosis (EMS) uses dissected temporalis muscle from the side of the head, where it is placed onto the surface of the brain through a burr hole in the skull. New vessels can then form between the muscle and brain, thus increasing blood flow [94]. A hybrid method called encephalo-duro-arterio-myo-synangiosis (EDAMS) uses both EDAS and EMS interventions to improve cranial perfusion. Alternatively, other tissue types such as omentum (lining surrounding the abdominal organs) and the frontal pericranial flap [95] or a multiple burr hole technique have been introduced for the development of angiogenesis [87]. Despite relatively few reports, neovascularization with these techniques have been observed [96-99].

While ischemia is the most common sign of moyamoya, hemorrhages occur in approximately 30% of adult patients [100]. Surgical treatment for hemorrhage, as in any patient presenting with bleeding, commonly include intraventricular drainage, craniotomy, and stereotactic aspiration [101]. Interestingly, bypass surgery as mentioned above may also help reduce the rate of recurrent hemorrhagic stroke [102]. Ding et al. state in a meta-analysis of articles reporting on 1050 patients, that revascularization intervention may be an optimal strategy to reduce hemorrhage in moyamoya patients [102]. Since decreased basal perfusion appears to be associated with recurrent bleeding in moyamoya patients, rectifying the

perfusion abnormality may prove beneficial [101]. While these treatment options can improve patient survival, a better understanding of moyamoya pathogenesis is needed to find treatment options that are less invasive and possibly reverse ICA stenosis.

Case Scenarios

It is vital to understand these cellular and biochemical changes in the context of clinical case scenarios. These cases highlight the variety of presenting symptoms, vasculopathy severity, and treatment options for people suffering from moyamoya.

Case 1: Hemorrhagic Presentation

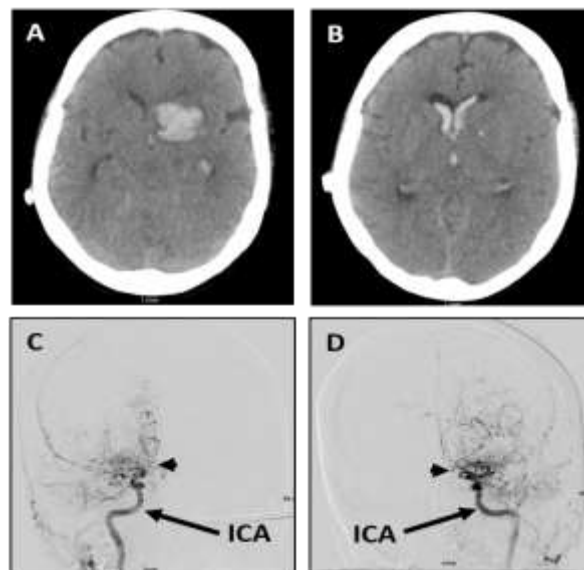


Figure 1. Non-contrast CT scan shows left caudate intracerebral hemorrhage (A) and intraventricular hemorrhage (B). Right (C) and left (D) AP views on digital subtraction angiography demonstrate bilateral moyamoya with occlusion of the supraclinoid internal carotid arteries (ICA; arrow) and robust lenticulostriate collateral formation (arrowhead).

A 54 year-old female with a known history of MMS and prior ischemic strokes, intraventricular hemorrhage, and ventriculoperitoneal (VP) shunting presented to our institution for the first time after becoming unresponsive in a witnessed incident. She was intubated on-scene, and brought to the emergency department, where her neurological exam was: awake, intubated, opens eyes to voice, localizing bilaterally, with some notable weakness of the right side. Non-contrast head CT showed a unilateral left basal ganglia intracerebral hemorrhage (ICH; Score 2) with intraventricular hemorrhage (IVH; Figure 1A and 1B). In addition, she was found to have a VP shunt infection. Ventriculostomy was placed and the VP shunt was removed. Cerebral angiography showed bilateral Suzuki stage VI MMS (Figure 1C and 1D). After 14 days of antibiotics and CSF drainage, her ventriculostomy was converted back to a VP shunt once CSF cultures were negative. This case illustrates not only a hemorrhagic presentation, but also some chronic complications of

moyamoya. The patient had previous disability related to multiple prior infarcts and hemorrhages, and she suffered a known complication of ICH/IVH; she had a VP shunt secondary to hydrocephalus caused by the ICH/IVH. Furthermore, she presented with a concomitant CSF infection, a known risk of VP shunts. Thus, this case demonstrates the true complexity of long-term management of moyamoya patients.

Case 2: Ischemic Presentation

A 36 year-old female with a history of migraine headaches, hypertension, and recent urinary tract infection presented with acute left arm weakness and numbness in the setting of a headache. Her NIH Stroke Scale on presentation was 3, and MRI demonstrated small scattered ischemic infarcts in the right watershed region (Figure 2A). Digital subtraction angiography (DSA) demonstrated bilateral Suzuki stage III MMS (Figures 2B and 2C). The patient underwent indirect bypass with EDAS and follow-up DSA 14 months later demonstrated robust collateral formation (Figures 2D-2G). This case highlights some vital concepts in moyamoya. First, not all strokes related to moyamoya are devastating and large; some are particularly small and focused along the cerebral watershed, a border zone between the tissues supplied by the anterior, posterior and middle cerebral arteries. As such, moyamoya can mimic complex migraine headaches. Second, as shown on the follow-up angiograms, the brain is ‘primed’ for neovascularization to feed the vascular requirements for adequate perfusion. Even with indirect bypass techniques, robust moyamoya collaterals still form.

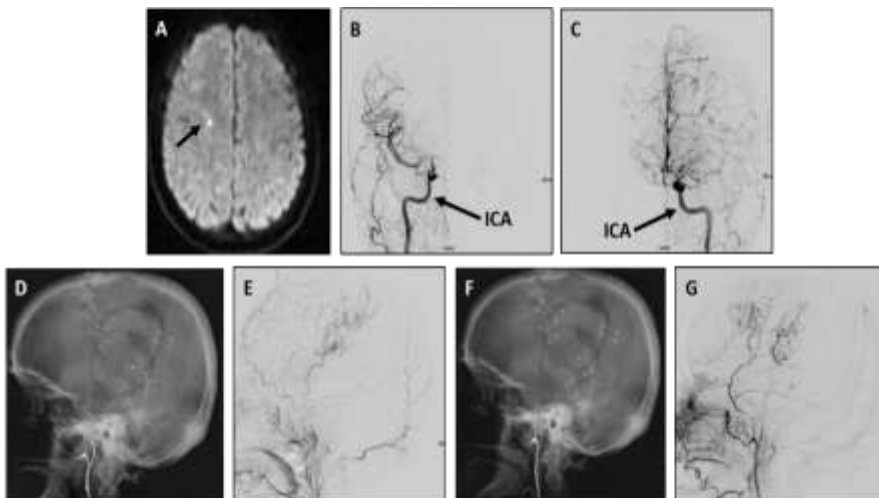


Figure 2. Diffusion-weighted MRI (A) shows small punctate infarcts along the ACA-MCA watershed (arrow). Right (B) and left (C) internal carotid artery injections on AP view demonstrate bilateral moyamoya. The patient underwent successful indirect bypass via the superficial temporal artery and middle meningeal artery with robust collateralization seen on the right [(D) unsubtracted and (E) subtracted] and left [(F) unsubtracted and (G) subtracted].

ANIMAL MODELS

One of the best ways to examine mechanisms that lead to the pathogenesis or progression of disease is with the use of experimental animal models. Animal models can undergo similar radiologic imaging currently used to diagnose moyamoya and can be used to study the effects of inflammation on blood vessel function. Furthermore, these models can be used to study the effects of moyamoya on cognition, an increasingly recognized chronic impairment in moyamoya patients. Although the etiology of MMD remains unknown, attempts to establish animal models are underway. Since chronic inflammation is thought to be a factor, studies which induce immunological reactions in the blood vessels of animals have been performed [103-105]. All of these studies resulted in alterations to the terminal ICA, including duplication of the elastic lamina, degeneration of media, and intimal thickening, as observed in moyamoya. However, the changes were minimal and were not sufficient to induce arterial stenosis, the major component of the disorder.

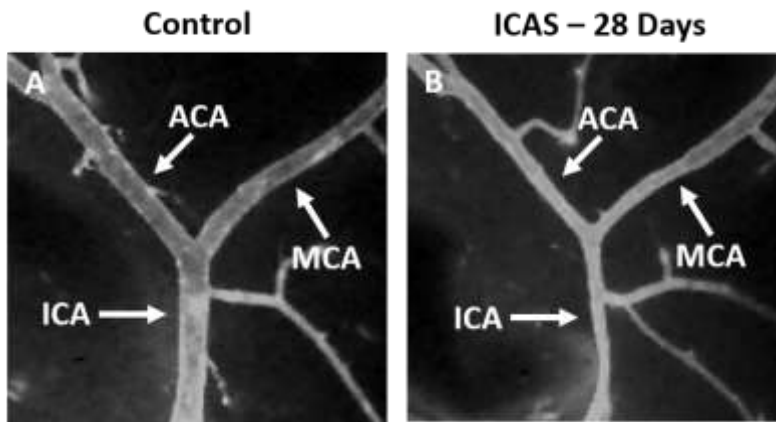


Figure 3. Images of Di I perfused brains from control (A) and ICAS (B) treated mice show decreased vessel diameter at the Circle of Willis 28 days after ICAS surgery. The internal carotid artery (ICA) bifurcates into the middle cerebral artery (MCA) and anterior cerebral artery (ACA).

As mentioned above, recent genome-wide and locus-specific association studies identified RNF213 as an important MMD susceptibility gene. A polymorphism in this gene was identified in 95% of familial and 79% of sporadic Japanese MMD patients [19]. Therefore, genetically engineered mice lacking RNF213 were generated by homologous recombination [106]. The authors performed magnetic resonance angiography (MRA), examined the anatomy of the Circle of Willis, and sectioned and stained the blood vessels to examine structural differences between the RNF213 knockout (RNF213KO) mice and wild-type littermate controls. Overall, there were no significant differences in the MRA, anatomy, or vascular wall thickness between groups out to 64 weeks of age. The authors report the RNF213KOs did not sufficiently induce MMD and recommend additional insults (inflammation, autoimmune response, ischemia) may be necessary for the development of MMD [106]. To address this question, Ito et al. performed transient MCA occlusion (tMCAO) on RNF213KO mice. Unfortunately, they did not find any differences in infarct volume, edema, or vascular density compared to wild-type controls. Interestingly, they did

report enhanced hind-limb angiogenesis and improved ambulation in the RNF213KO mice following hind-limb ischemia [107]. While RNF213 may be involved in the development of peripheral vascular networks following injury, its role in the vasculature of the brain remains unclear. To complicate matters, Sato-Maeda et al. report an upregulation of RNF213 in neurons following tMCAO. The increased expression of RNF213 mRNA co-localized with TUNEL-positive (apoptotic) neurons within the ischemic penumbra, suggesting RNF213 may be involved in either cell death or attempted survival following injury [108]. Further investigation is required to clarify the role of RNF213 in the pathophysiology of MMD.

While MMD is thought to have a genetic component, MMS is sporadic and associated with other well characterized co-morbidities. To examine the pathogenesis of MMS, we developed the ICA stenosis (ICAS) surgical procedure in which a micro-coil was applied unilaterally to induce hypoperfusion in mice [109]. We report a significant decrease in the diameter of the distal ICA (Figure 3), as well as a decreased number of cortical anastomoses in the watershed region, both consistent with MMS. While the pathology observed after 28 days is consistent with an early Suzuki stage and more extensive evaluation is needed, this methodology could provide a key tool for studying MMS in the laboratory. Specifically, this model can be employed in a variety of co-morbid animal models to investigate the effect disorders may have on MMS development and progression.

CONCLUSION

In conclusion, moyamoya is an increasingly recognized cause of stroke in both children and adults. As more varied populations present with moyamoya across the world, there is a growing need to delineate syndrome from disease, as factors that influence vasculopathy development may differ between the two. Inflammation could be a key player in the development and progression of moyamoya pathology, though additional studies are needed to determine the exact role of the immune system in both MMD and MMS. The use of newly developed animal models are critical to our understanding of these pathologies and for the possible development of new treatment strategies. While revascularization interventions provide temporary benefit, extensive epidemiological, genetic and co-morbid disorder research will need to be undertaken to fully understand this phenomenon and how best to treat those patients that suffer from it.

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Chapter 83

THE COMBINED USE OF tDCS AND OTHER REHABILITATION TECHNIQUES TO IMPROVE UPPER LIMB MOTOR FUNCTION AFTER STROKE

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ABSTRACT

Transcranial Direct Current Stimulation (tDCS) has recently gained a lot of attention as a tool to modulate neuronal plasticity influencing motor learning processes and functional recovery after stroke (Schlaug & Renga 2008; Chisari et al. 2014). The main conceptual target is to use tDCS for normalizing imbalanced inter-hemispheric inhibition, increasing excitability of perilesional intact regions of the affected hemisphere with anodal stimulation and/or decreasing excitability of the contralesional hemisphere with cathodal stimulation (Celnik et al. 2009; Fregni et al. 2005; Hummel et al. 2005; Hummel & Cohen 2005; Hummel et al. 2006). First evidences demonstrated transient improvement of upper limb motor function after a single session of tDCS and some studies have suggested potentially cumulative effect of multiple sessions of tDCS in improving motor learning in healthy subjects and hand motor recovery in patients with stroke (Boggio et al. 2007; Reis et al. 2009). These findings allow to support the hypothesis that combining multi-sessions tDCS protocol with other upper limb rehabilitative techniques which use principles of motor learning in clinical settings could be optimal to improve upper limb motor recovery after stroke (Wessel et al. 2015; Nair et al. 2008).

At this purpose we overviewed studies in which a combined upper limb rehabilitative therapy including tDCS was administered to subacute or chronic stroke patients. Different treatments were associated with tDCS such as virtual reality-based therapy (VRT), robot assisted arm training (AT), constraint induced therapy (CIMT), or occupational therapy (OT). First evidences revealed that tDCS sessions can be administered simultaneously to conventional therapy sessions, and the mean tDCS treatment duration was about 10-15 sessions for 2-3 weeks. Most of these studies showed significant effects for experimental treatment groups versus control groups, evaluated with different clinical scales for upper limb motor function. All studies used functional scales to explore upper limb motor improvement, but e.g., Bolognini et al. (2011) also

showed a neurophysiological correlate to identify reduction in inter-hemispheric inhibition probably mostly due of tDCS effects. Particularly effective was the association between tDCS and OT or CIMT (Nair et al. 2008; Lindberg et al. 2012; Bolognini et al. 2011). Conversely other types of combined trials showed no significant or only preliminary results in upper limb outcome measures, probably due to a very small sample size.

In this overview we conclude that tDCS can be considered an important tool to maximize the effects of traditional therapies for upper limb motor recovery, modulating the plasticity-dependent processes after stroke (Wessel et al. 2010). However it will be useful to clarify the timing and duration of tDCS administration protocols and how these parameters influence other therapies effects, in order to identify a useful combined upper limb rehabilitation strategy for stroke patients.

INTRODUCTION

Upper limb motor impairment is common in patients after stroke. It causes an important restriction in daily activities and strongly impacts on quality of life (Carod Artal E et al. 2009; Kwon SU et al. 2000). Currently stroke rehabilitation strategies are developed on increasing scientific and clinical evidences based on neural remodeling after brain damage and neuroplasticity mechanisms (Kleim et al. 2008).

It is now widely known that neuroplasticity is the property of the nervous central system to modify and rearrange neural connections following injury or environmental changes and it is not only an occasional state but the normal ongoing state of the nervous system throughout the life span (Cramer et al. 2011; Chisari et al. 2014).

Combined Therapies for Upper Limb Motor Recovery

Transcranial direct current stimulation (tDCS) represents the noninvasive version of direct current application to the scalp and it modulates cortical activity by constantly applying weak electrical current over time to increase or decrease cortical excitability (Nitsche et al. 2008). In stroke rehabilitation this means that anodal tDCS (A-tDCS) may be employed over the affected hemisphere to improve the response of that hemisphere to training protocols, or on the other side, cathodal stimulation (C-tDCS) may be used to inhibit the unaffected hemisphere in order to reduce trans-hemispheric inhibition (Hummel et al. 2005). In addition tDCS can be used applying bihemispheric stimulation that contemporary modulate the unaffected and affected motor cortex (Dual-tDCS) (Nitsche et al. 2003; Lindberg et al. 2010). About this a great consideration should be given to the placement of the electrodes and the focality of tDCS interventions. Different electrode configurations may result in different patterns of current spreading over the scalp and consequently on the cortex. It is feasible that the typical “reference” position over the supraorbital region may produce undesired stimulation in non-target regions, thus newer monopolar stimulation montages attempt to avoid this problem (DaSilva et al. 2011). While substantial work is under way to model the fields induced by these different montages, clear behavioral or physiological data is lacking on the differences between these approaches. A lot of studies explored tDCS effects on healthy subjects and the most commonly studied brain area is the left M1. For M1 modulation

active electrode is placed over the representative field of the right hand, whereas the reference electrode is generally placed on the contralateral supraorbital region (Nitsche et al. 2008). Lots of these studies demonstrated that tDCS applied over M1 influences cortical connectivity with more evident results (measured by EEG or fMRI) during voluntary hand movements than during rest (Polania et al. 2011; 2012). In particular Polania et al. (2011) showed an increased synchronization in alpha and lower frequency bands in frontal and parieto-occipital regions, and in the high gamma frequency band in motor related regions during voluntary hand movements and with fewer changes in during rest. Another work demonstrated that anodal tDCS over left M1 during rest only increased the power density of low frequency oscillations (theta, alpha) (Pellicciari et al. 2013). These results suggest that major changes in brain activity associated with tDCS are augmented by its combination with performing an active behavioral task (Fritsch et al. 2010). All these evidences allowed to think that coupling an hand task or a different treatment for upper limb with tDCS in stroke patients could have a synergic effect on motor recovery. So far tDCS has been used as a treatment to enhance motor recovery after stroke, but it has been mostly examined without or independently of a specific rehabilitation treatment and its effects have been evaluated in term of functional improvement with neurophysiological measures (Bolognini et al. 2014). Another important aspect of tDCS administration is treatment duration: it is not clear until now if a multisession protocol leads to longer lasting and more evident effect compared to single sessions of brain stimulation. Single sessions of tDCS showed only short-lasting and variable effects on cortical excitability and performance both healthy subjects and stroke patients (Nitsche et al. 2008). Recent studies reported positive effects of tDCS treatments on activities of daily living (ADL) and on upper limb motor function. They showed evidence in favor of tDCS short-term effects compared with controls at the end of the intervention phase (in terms of Fugl-Meyer upper limb score), but not at three months follow-up (Elsner et al. 2013). Effects of combined treatments were also analyzed in other studies in which an upper limb (UL) rehabilitation treatment was associated with tDCS sessions (Tedesco Triccas et al. 2015). Wide variability in protocols, patients' upper limb motor impairment and different timing of treatment administration made difficult to understand the results. In the following text we cited studies in which a combination of tDCS and upper limb treatment was applied in stroke patients. First of all, in all studies chronic stroke patients were enrolled, except for one paper (Lee and Chun 2013) in which the intervention was applied in subacute post-stroke phase. In addition limited details were reported about stroke location and lesion size. However the majority of these studies declared that participants had moderate UL impairment. A wide variability has been showed in tDCS protocols even for stimulation time, current intensity, electrode size, and type of stimulation. Bihemispheric stimulation was applied in two of these studies (Bolognini et al. 2011; and Lindberg et al. 2012), anodal stimulation in one (Viana et al. 2014), anodal plus cathodal stimulation without sham in another one (Ochi et al. 2013), and cathodal stimulation protocol in the last two (Lee et al. 2014; Nair et al. 2011).

tDCS + Constraint Induced Movement Therapy

Constraint-induced movement therapy (CIMT) is a stroke rehabilitation approach that involves the forced use of the affected arm by restraining the unaffected arm (Corbetta et al. 2010). Bolognini et al. (2011) conducted a randomized control study in which 14 chronic stroke patients with a moderate to severe hemiparesis (Upper Extremity Fugl-Meyer, UE-FM, mean score 26) were randomized between a combined CIMT and bihemispheric tDCS group

and a sham group. Each subject underwent a 14-day CIMT up to 4 hours per day administered by a trained therapist. They had to wear a resting splint secured in a sling, which hindered hand and finger movements and they had to complete 9 different shaping tasks. Bihemispheric tDCS was applied during rehabilitation sessions to study group and stimulation time was about 40 minutes, while control group received sham tDCS (that consisted in a 30 sec of stimulation) and CIMT. They collected functional outcome measures at baseline, day 1, day 5, at the end of the treatment and at 2 and 4 weeks follow up. They used qualitative scales as Jebsen Hand Function Test (JHFT), Motor Activity Log Rating Scale (MAL) and quantitative evaluations as Fugl-Meyer (FM), Handgrip Strength (HS) for upper limb activity. They also evaluated neurophysiological parameters for cortical excitability assessment such as motor evoked potential (MEP) and transcallosal inhibition (TI) in both cortices. As it concern motor function the treated group showed an improvement in UE-FG score and HS at the end of the treatment that was absent in sham group. Qualitative scales didn't show significant difference between the two groups. Active group also showed increased mean peak-to-peak amplitude of MEPs after the treatment, not evident in sham group. Changes in cortical excitability were correlated to motor improvement. A positive correlation was found in MEPs amplitude of the affected hemisphere and FM score. Conversely negative correlations were found between intact-to-damaged TI and the FM score and the JHFT. They demonstrated that bihemispheric tDCS increased the effects on upper limb motor function induced by CIMT as assessed with JHFT, HS and FM. They concluded that tDCS bring about a more favourable interhemispheric balance. This study described subjects and treatment characteristics in details and upper limb motor impairment with multiple evaluation scales were analyzed interpreting results with a neurophysiological correlation.

tDCS + Virtual Reality Therapy

Virtual Reality Therapy (VRT) is a computer-based technology that permits the interaction with a multisensory simulated environment and allows to receive a real-time feedback on motor performance. Exercises in VRT have the potential to apply relevant concepts of neuroplasticity (Langhorne et al. 2009). Viana et al. (2014) conducted a RCT on chronic stroke patients randomized in two groups: one for a combined 5 weeks treatment composed by virtual reality therapy (Wii gaming System) and anodal tDCS, and control group with VRT and sham tDCS. They enrolled 20 patients with mean age of 56 ± 12.2 years with unilateral ischemic (except 1 hemorrhagic) stroke within the previous six months. No data have been cited regarding lesion location. Baseline assessment has been conducted with Mini Mental State Examination (MMSE) for cognitive profile and with FM, Wolf Motor Function Test (WMFT), Modified Ashworth Scale (MAS), Grip strength for quantitative motor assessment and Stroke Specific Quality of Life scale (SSQoL) as qualitative tests. Patients were homogeneous for clinical and functional characteristics and they were included only if they were able to hold Wii controller. This type of treatment determined a straight selection of patients because they didn't have to present cognitive impairment, with a mild-to-moderate upper limb motor impairment. Treatment consisted in 3 times for 5 weeks, one hour per session of Wii console games, 3 games with different tasks associated for experimental group with anodal tDCS sessions. It was not declared if stimulation occurred during or far from the Wii performance. Outcome measures were collected at the end of treatment. Both groups experienced after treatment similar improvement underlined through changes in the FM-UL

and WMFT scores, with no significant difference between them. Even though the hypothesis of this study was to associated the previous demonstrated beneficial effects of 15 hours VRT on upper limb motor rehabilitation (in particular on ADL) with the beneficial of tDCS treatment, this combined treatment didn't demonstrate an increased positive effect on UL motor recovery. However they showed that only experimental group revealed decrease tonus of the wrist flexor muscles, and this evidence was supported by other tDCS study (Wu et al. 2012). A different associated therapy was tested in one of the 3 study groups conducted by Lee et al. in 2014. In this case they tested subacute patients (within 1 month after stroke). Subjects were randomly assigned to this experimental group who received combined therapy with cathodal tDCS administered during 15 VRT sessions (30 min/d, 5 times/wk for 3 weeks) or to one of the other study groups. Cathodal tDCS was performed placing the electrode on unaffected hand motor area with an intensity of 2 mA. Virtual reality system used for UL rehabilitation consisted in a monitor, video camera and computer that recognized the system's gloves and virtual objects, able to recognize the patient's movement and position and transfer subjects into the virtual space. Each patient, viewing him/herself into the virtual scene, must performed three different reaching and trajectory programs using the affected arm. Twenty patients tested this type of treatment and results were compared with other two study groups which experimented tDCS and occupational therapy or only VRT. Patients in three groups were homogeneous for gender and age (mean 60+/-13) and also for lesion type (hemorrhagic/ischemic) and location (cortical/subcortical). They were tested before and after treatment and all groups demonstrated a significant improvement in clinical scales at the second assessment. However combined treatment with tDCS and VRT group showed significant improvement in Motor Function test and FM compared with other groups and the latest was significantly higher. In conclusion these results supported a synergic effect of two different treatments on motor recovery after stroke. In particular patients enrolled in this study had no severe UL motor impairment and MEP were present. The combination of brain stimulation using tDCS and peripheral arm training using VRT could provide additional benefits to the training of UL recovery after stroke over those achieved with either intervention alone. This paper differs from the others because authors described an early treatment in subacute stroke patients and it will have a good impact on motor recovery. In addition all subjects received conventional rehabilitation including physical, occupational and cognitive therapy with the same intensity and time, but it is a potential risk of bias that could implement rehabilitation effects.

tDCS + Robot Assisted Arm Training

Robotic devices allow users to participate in interactive training and motor relearning via high-intensity, repetitive, and frequent tasks (Peter et al. 2011; Basteris et al. 2014) and were recently widely used in rehabilitation trainings. This kind of combined treatment was tested by Ochi et al. (2013) on 18 chronic stroke patients. All patients received two interventions, comprising either anodal tDCS to the affected hemisphere with Robot Assisted Arm Training (AT) or a cathodal tDCS to the unaffected hemisphere with AT for 5 days in a cross over manner. The two interventions were separated by a 2 days period. Patients had a long-term, moderate to severe motor impairment of an upper limb from a first stroke that had occurred over 6 months before. In this case 11 patients experimented an hemorrhagic stroke and 7 an ischemic ones. Upper limb treatment was performed with the Bi-Manu-Track robotic arm trainer, which permitted a mirror-like practice of two movements cycles: forearm pro-

supination and wrist flexion-extension. Patient grasped with both hands a handle with a strap holding the paretic hand in place. Within one session subjects practiced 500 forearm cycles and 500 wrist cycles for a total of 1000 cycles in 2 different modalities: passive-passive in which both arm were moved by the robot and active-passive with the unaffected arm driving by the affected side. Direct current stimulation was delivered at 1 mA for the first 10 minutes during AT. Clinical evaluations were performed before and after the two training sessions with FM-UL, MAS and MAL. The final analysis showed that there were small but significant improvement effects on FM-UL and MAS in both trainings, but not in MAL. In particular there were no significant effects comparing the first and the second stimulation period, and only the cathodal stimulation and AT was associated with an improvement in MAS for fingers. This evidence supported the previous results even in chronic patients of anodal tDCS and AT combined treatment showed by Hesse et al. (2011) on subacute stroke ones, but also added similar results for cathodal stimulation of the unaffected hemisphere. However in this case there wasn't a sham group to compare the no-treatment effect and patients enrolled were very heterogeneous even for type of lesions.

tDCS + Occupational Therapy

This type of combined treatment was tested, compared with a sham treatment, on fourteen right handed stroke patients by Nair et al. (2011). The experimental group contained 5 patients with predominantly cortical lesions including the immediate underlying subcortical regions and 2 with deep white matter/striato-capsular regions. In the sham group 4 subjects had a predominantly cortical lesion and 3 white matter/striato-capsular lesions. Subjects, aged between 20 and 76 years, were randomized between a 5 days cathodal tDCS and OT group and a sham tDCS and OT group. Stimulation consisted in 1 mA intensity current applied during the first 30 minutes of one daily hour of occupational therapy, focusing on proprioceptive neuromuscular facilitation techniques. In this case patients were evaluated at baseline with Range-of-motion (ROM) measure for shoulder abduction, elbow and wrist extension and with FM-UL and these measures were repeated after one week to the end of treatment. They also employed a fMRI evaluation in which has been request to the patient to performed one of 2 motor tasks that could be: a full wrist extension and flexion or a full elbow flexion and extension up to the maximum excursion (if the patient was not able to perform the first). In this case all subjects had a moderate to severe upper extremity impairment and patients in experimental group showed slight differences to the disadvantage in terms of mean age, time to stroke and lesion size (even if these differences were not significant). However this study showed that the combined therapy enhanced UL motor function in chronic stroke patients with moderate to severe motor impairment, more than similarly intense protocol of sham tDCS and OT. These results were supported by ROM and FM-UL outcome measures that were significantly greater in experimental group after the treatment. In addition fMRI was used to analyze changes in activation in precentral gyrus region, demonstrated that cathodal tDCS applied to the contralesional hemisphere resulted in a reduced activation level, presumable referring to a decrease of the abnormal excitability levels in that hemisphere. This evidence was supported by the finding of an inverse correlation between changes in contralesional activation and a positive progression in the FM-UL score. A variant of this combined treatment was used by Lindberg et al. (2012), that tested a double 5 sessions treatment compared with a sham treatment on 10 chronic stroke patients. They used a combination of 30 minutes bihemispheric tDCS during one hour of upper limb

therapy. Upper limb therapy consisted in a combination of physical therapy (PT) and OT techniques, including functional motor tasks of the affected arm and hand to promote coordination of movement, goal-directed strategy and sensorimotor integration. Therapists administered the first 5 days session and after a period of 2-29 day a second session of 5 days rehabilitation. Patients enrolled, mean age 50,3 $\pm$ 15,2, experimented first ischemic event and they were heterogeneous for lesion size and location. WMFT and FM-UL assessment were done at baseline for each 5 days-session and after the first and the second rehabilitation period. Results reported that changes were greater during the first intervention than the second for both outcome measures, and mean improvement in the experiment group was substantially greater compared with the sham. However results obtained after the second treatment period were lower than those observed during the first. It's difficult to compare this kind of rehabilitation protocol with the others cited below, because it was administered in two different times with a variable interval between subjects. They also enrolled a very small and heterogeneous sample size that could confound results.

Comments

From a general point of view, these studies demonstrated that multisession tDCS protocol could be safety and easily applied even simultaneously combined with other rehabilitative approaches for stroke patients. However systematic reviews and meta-analyses showed that multiple sessions of tDCS regimens combined with UL rehabilitation had a small and no-significant effects on upper limb impairment (Tedesco Triccas et al. 2015). Actually tDCS has not yet been applied in clinical practice as a rehabilitative treatment and as it showed in previous paragraphs, there is still a wide variability in tDCS protocols applied in stroke patients. Therefore it is unclear what kind of UL rehabilitation treatment can be synergic, useful or non-influent in association with a central stimulation intervention. Probably these small evidences can be due to a lot of difference, not only in protocols used but also in patients' clinical characteristics, lesion type and grade of upper limb motor impairment. However, given this variability, a "one size fits all" approach is not to be considered optimal for stroke rehabilitation (Gary et al. 2015). Accordingly, there is a great interest in identifying appropriate markers in order to obtain a specific targeted rehabilitative treatment that combines central and peripheral stimulation.

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Chapter 84

DOES ANOSOGNOSIA AFFECT THE SHORT-TERM GOALS ACHIEVEMENT IN MEDICAL REHABILITATION FOR PATIENTS WITH ACUTE ISCHEMIC STROKE?

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ABSTRACT

Insufficiency of self-awareness for neurological deficits may negatively affect the functional outcomes in medical rehabilitation due to the decreased engagement of the patients in the rehabilitation process [1-4]. However, its relationships with rehabilitation goals attainment in the patients with acute ischemic stroke (IS) have not yet been clarified.

PURPOSE

To evaluate the impact of anosognosia on the short-term functional rehabilitation goals achievement in the acute ischemic stroke patients.

METHODS

We examined 57 patients (36 males, 21 females, mean age $64,8 \pm 9,2$ years) in the beginning of acute period of ischemic stroke (mean time since IS = $4,8 \pm 1,1$ days) and then 2 weeks later.

Inclusion criteria were: acute period of ischemic stroke; neurological and cognitive deficit interfering with function. Patients were excluded if they had severe cognitive

impairment (dementia), aphasia or concomitant illness that prevented the understanding of instructions and active participation in rehabilitation process.

The assessment consisted of clinical neurological examination, neurovisualisation (CT/MRI), use of Frontal Assessment Battery (FAB) [5] and several assessment methods aimed at detecting of anosognosia. We used our own method of evaluation of unawareness for motor and cognitive abilities in brain damaged patients (EUMCA) as well as Russian version of Dysexecutive Questionnaire (DEX) of the Behavioural Assessment of the Dysexecutive Syndrome (Wilson B.A. et al, 1996; BADS Russian Translation 2009 by Pearson. All rights reserved) and Anosognosia Score for Hemiplegia [6,7].

EUMCA comprises two parts, one to assess inadequacy of self-rating of motor abilities (MA), and second to assess inadequacy of self-rating of cognitive abilities (CA). Each part is based on a comparison of predicted and actual performance of a set of simple tasks in motor and cognitive areas, respectively. Perception of self-capabilities was measured using two questionnaires. Each of them included 10 questions for self-appraisal of daily life activities. Actual performance was assessed using two objective rating subscales for the same tasks that were asked about in the questionnaires. The cognitive tasks were addressed to executive, visual-spatial and memory functions. We have previously shown that both questionnaires and both objective rating subscales had acceptable psychometric characteristics according to Rasch analysis. This allowed to measure unawareness for motor or cognitive abilities as the discrepancies between self- anticipated and observed performance of motor or cognitive tasks. Discrepancy scores both for motor tasks and cognitive tasks had a normal distribution so they were converted into standardized scores (z-scores) on the basis of normative data. The standardized scales for evaluation of unawareness for motor abilities (UMA) and cognitive abilities (UCA) were created. The criterial validity of the proposed scales has been verified by applying of DEX at the previous stages of work. The discrepancy score that exceeded the average value on a standardized scale more than for two Z- scores was taken as a criterion of pathological overestimation of motor abilities ("anosognosia for motor deficit") and as a criterion of pathological overestimation of cognitive abilities ("anosognosia for cognitive deficit"), depending on the used set of tasks.

The patients underwent a 2-week multidisciplinary early task-oriented rehabilitation. In case of anosognosia the rehabilitative specialists purposefully captured the patient's attention to his/her neurological deficit to increase the awareness of this deficit and to involve the patient in the rehabilitation process. Individual short-term rehabilitation goals in the domains of impairment, mobility and activities of daily living were formulated for each patient and agreed with him/her at the initial team meeting. In this study we analyzed the degree of achievement of goals only in the domains of mobility and daily activity ("function" goals) because the regress of impairment to a large degree occurs spontaneously in the acute period of stroke. The achievement of functional goals was scored prior to the rehabilitation in accordance with Goal Attainment Scaling [8]. There was no hierarchy of goals, and all goals were given a weight of «1». Indicators of improvement, or lack of it were identified in behavioral terms. The expected level of outcome was specified for each selected goal and a primary value of "0" was assigned to it. Baseline primary scores for each goal were rated as "-2". Primary scores for "somewhat less than" expected levels of each outcome were rated as "-1", where scores "0" and above implies the full goal achievement.

Functional improvement was measured in 2 weeks using Goal Attainment Scaling as an indicator of success. Rating of individual goal attainment was undertaken at follow-up in 12

days according to the initial specifications for outcome levels. The aggregated composite T-score of each participant's goals attainment was calculated as recommended by S.Gauggel and V.Hoop, 2004 [9]. Median achieved T-scores were 50. Scores above 50 indicated a better outcome than expected; scores below 50 indicated a worse-than-expected outcome.

The degree of final goal attainment of the patients was graded as a complete goals achievement (CA), incomplete achievement (IA) and lack of achievement (LA). Exceeding of the planned goals was also evaluated as "CA". The absolute lack of achievement of the patient's selected goals was diagnosed if the final aggregated T score corresponded to the baseline "-2" level for each target, i.e., to the baseline level of the patient's abilities.

Descriptive statistics were used to summarize patient's characteristics which were described as frequency and percentage, median and range. Groups were compared by Fisher's exact test. To assess the baseline UMA scores and UCA scores in CA, IA, LA patients, the nonparametric test of Kruskal-Wallis was used. The difference between two groups were analysed with U Mann-Whitney test. Correlation between variants was assessed with the use of Spearman's rank correlation coefficients. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

The patients were divided into three groups in accordance with the results of EUMCA, DEX and Anosognosia Score for Hemiplegia: group 1 (without any anosognosia, 15 patients), group 2 (anosognosia for cognitive deficit only, 24 patients) and group 3 (anosognosia for both cognitive and motor deficit, 18 patients). Pathological overestimation for motor abilities coexisted with pathological overestimation for cognitive abilities in all cases. In other words, there were no patients with "isolated" anosognosia for motor deficit, i.e., without coexistence with anosognosia for cognitive problems.

The patients in the third group had right-sided hemispheric ischemic stroke (16/18 or 89%) more often than the patients in the first group (7/15 or 47%), $p = 0,048$, and in the second group (16/24 or 67%) $p = 0,004$. Involvement of a right parietal lobe was also more frequent in the third group (13/18 or 72%) than in the first (2/15 or 13%), $p = 0,006$, and in the second group (8/24 or 33%), $p = 0,0003$. At the same time, there were differences between the 1, 2 and 3 groups in FAB results ($F = 23,95$, $p = 0,0000001$). FAB score in patients without any anosognosia ($15,4 \pm 1,7$) was higher than in the patients with cognitive anosognosia only ($11,1 \pm 2,8$), $p = 0,000005$ and in the patients with anosognosia for both cognitive and motor deficit ($9,1 \pm 3,0$), $p = 0,03$. The patients with "isolated" cognitive anosognosia had the higher FAB score in compare with the patients with anosognosia for both cognitive and motor deficit. In the whole group of observed patients FAB score negatively correlated with UCA ($r = -0,53$, $p < 0,05$) and with UMA ($r = -0,40$, $p < 0,05$). In other words. the better the function of the frontal lobes, the less overestimation of cognitive and motor abilities in the acute stroke patients.

CA of the individual functional rehabilitation goals was found in 18 patients on the final evaluation, while IA and LA were identified in 24 and in 15 patients, respectively. The proportion of patients without any progress in achieving the goals toward which one has worked, was statistically higher in the patients with baseline anosognosia for both cognitive

and motor deficit, i.e., in the third group (10/18 or 55,6%) than in the first group 1 (2/ 15 or 13,3%) and second group (3 /24 or 12,5%), $p = 0,006$ and $0,001$, respectively. There were no statistically significant differences in the frequencies of IA and CA between the first and the second groups.

The assessment of the baseline UMA scores in CA, IA, LA patients demonstrated statistically significant interaction between the baseline unawareness for motor abilities and the degree of the goals achievement ($F = 3,96$, $p = 0,025$). In patients who finally did not achieve the rehabilitation goals, the baseline mean UMA Z score ($+2,8$ [$+1,0$; $+3,4$]) indicated the pathological overestimation of motor abilities (“anosognosia for motor deficit”). In the patients who completely or partially achieved the rehabilitation goals, the baseline median UMA Z scores were within the normative range. The mean baseline UMA Z-score was higher in LA patients in compare with the IA patients ($+1,0$ [$-0,3$; $+2,0$]), $p = 0,031$, and the CA patients ($+1,2$ [$-0,2$; $2,0$]), $p = 0,06$.

Similarly, there were differences in the initial UCA scores in the CA, IA and LA patients ($F = 5,67$, $p = 0,006$). The baseline mean UCA Z score ($+3,1$ [$2,4$; $4,6$]) in the patients with lack of goals attainment designated the pathological overestimation of cognitive abilities (“anosognosia for cognitive deficit”). It was higher in the LA patients than in the IA patients ($+2,4$ [$+1,0$; $+2,6$]), $p = 0,002$, and that in the CA patients ($+2,4$ [$+1,4$; $+2,8$]), $p = 0,06$, respectively.

DISCUSSION

Anosognosia for both motor and cognitive deficit in our study was associated not only with the right parietal lobe involvement but also with the worse frontal lobe function. This assumption is supported by correlations between FAB scores, UCA and UMA scores that were found in our study. It is consistent with the data of A.De Carolis et al. (2015), who demonstrated the association between anosognosia and general cognitive deficit in nondemential patients. These authors concluded that anosognosia depends on the dysfunction of an extensive neural network including the frontal lobe structures [10].

The large number of patients (15/57 or 26%) in our study did not reach the intended rehabilitation functional goals at all. This can be explained by the fact that we evaluated progress only in the domains of mobility and daily activity, but not in impairment sphere. If we assessed also the goals in the domain of impairment, the results would be obviously better. The lack of goals attainment was observed most often in the patients with anosognosia for both cognitive and motor deficit than in patients without anosognosia or with anosognosia for cognitive deficit only. This fact agrees with studies indicated that the patients with anosognosia for hemiparesis do not feel the need to participate in rehabilitation and so have poorer functional outcomes [11-13].

The impact of anosognosia for cognitive deficit on the effectiveness of rehabilitation of stroke patients is less known. H.Boosman et al. (2014) found that overestimation of memory functioning may be present in stroke patients with a good functional outcomes [14]. However, the effect of unawareness of other cognitive problems on the results of medical rehabilitation has not been studied. The fact that the patients without anosognosia and with isolated cognitive anosognosia in our study did not differ in the frequencies of the lack of

rehabilitation goals achievement, complete goals achievement and incomplete achievement are consistent with data of H.Boosman et al. [14]. Our results suggest little influence of overestimation of cognitive deficit on the goals attainment in rehabilitation of patients without dementia.

However the baseline UCA scores in our study were higher in the patients who finally did not achieve the functional rehabilitation goals. Seemingly, it implies that pathological overestimation for cognitive abilities prevents the achievement of short term functional rehabilitation goals in the acute stage of ischemic stroke.

But there is no contradiction with the above results. The higher baseline UCA scores in the patients who failed to achieve the rehabilitation goals most likely were most likely due to the fact that part of the patients with pathological overestimation of cognitive abilities had also the pathological overestimation of motor deficit, which, in turn, was associated with worse functional outcomes. The negative influence of overestimation for both cognitive and motor abilities on the goals achievement may be mediated by frontal lobe dysfunction, which on the one hand predisposes to anosognosia, and, on the other hand, impairs the purposeful activity. This assumption is supported by correlations between FAB scores, UCA and UMA scores that were found in this study.

Further studies are needed to clarify whether the use of special techniques facilitating the regress of anosognosia (such as video-based self-analysis of errors) may improve the results of rehabilitation of the patients, pathologically overestimated their abilities.

CONCLUSION

The coexistence of pathological overestimation of cognitive and motor abilities has a negative impact on short term goals attainment in the early rehabilitation of acute ischemic stroke patients.

Isolated anosognosia for cognitive deficit has no significant influence on rehabilitation goals achievement in nondemential acute stroke patients.

Negative influence of anosognosia for both motor and cognitive abilities on the results of rehabilitation interventions may be mediated by dysfunction of frontal lobes which is connected with parietal lobe and other cerebral structures.

The presence of anosognosia for motor and cognitive deficit requires the review of the short term rehabilitation interventions for acute stroke patients.

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Chapter 85

VIDEO-BASED QUANTIFICATION OF PATIENT'S COMPLIANCE DURING POST-STROKE VIRTUAL REALITY REHABILITATION

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ABSTRACT

We present a video-based monitoring system for the quantification of a patient's attention to visual feedback during robot assisted gait rehabilitation. The patient's face and facial features are detected online and used to estimate the approximate gaze direction. This gaze information is then used to calculate various metrics of the patient's attention. Results demonstrate that such unobtrusive video-based gaze tracking is feasible and that it can be used to support the assessment of patient's compliance with the rehabilitation therapy.

Keywords: Video-based attention monitoring, gaze estimation, stroke rehabilitation, user compliance

INTRODUCTION

Stroke is the third most common cause of death in Western society, with about 4.7 million stroke survivors alive today. One of the hallmark residual impairments of stroke is post-stroke walking disability, which creates a stigma for patients, makes them more

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susceptible to injury and directly affects their quality of life. Early rehabilitation therapy is crucial for significant improvements (1). In recent years, robotic systems have been widely tested and employed to retrain stroke patients. By imposing gait-like movements at a comfortable speed, such robotic devices are thought to provide many of the afferent cues regarded as critical to the retraining of locomotion (2).

However, a major problem with existing stroke therapies is patient non-compliance (3). Many stroke patients abandon the therapy because the process is too long, repetitive, and/or does not provide immediate results (4). The European project BETTER (5) recently addressed a new approach to gait rehabilitation by employing non-invasive brain-neural computer interaction (BNCI) based assistive technologies based on EEG (Electroencephalography), EMG (Electromyography) and IMU (Inertial Measurement Unit) sensors. In this article, we extend the BNCI-based modalities with a video-based attention monitoring system that allows automatic quantification and long-term monitoring of user attention. Such attention tracking does not require any sensors to be attached to the patient, making this method easy and fast to apply in stroke rehabilitation. Our main objective is to quantify the patient's attention to visual feedback, i.e., the amount of time the patient's gaze is actively following the displayed visual feedback and to inspect the possible impact of visual feedback on motor planning, as well as its short- and mid-term benefits.

Several studies have already examined the impact of visual feedback on stroke rehabilitation, but they mostly reported inconclusive results. They focused on quantification of results of rehabilitation, enhanced with different kinds of visual feedback, but their experimental designs did not allow for a reliable quantification of the attention a person is paying to stimuli. To the best of our knowledge, only psycho-physiological measures of a patient's attention to visual feedback has been reported in (6), while video-based assessment of attention has not been proposed in the field of rehabilitation.

Methods for real-time detection of the face, facial features, eye movements and gaze direction from video have attracted a lot of research in the past decade (7). Numerous algorithms for facial feature extraction have been proposed, mostly in the context of face detection and recognition (8). Currently, the most promising approaches rely on fusing multiple visual cues, such as combining local feature matching with intensity-based methods (9). Existing methods for the quantification of user attention to visual feedback have mostly been developed for Human-Computer Interaction applications and in order to help severely disabled people (10).

METHODS

An overview of our approach is depicted in Figure 1. The patient is fixed in a robotic gait trainer, which moves the patient's legs according to predefined rehabilitation scenarios, and is adapted to the patient's current walking abilities. In front of the patient is a large TV screen showing various types of visual feedback. A high speed video camera is mounted above the TV. The camera captures HD video of the patient's face, while simultaneously EEG data are recorded from 51 scalp sites and EMG data are recorded from both legs (tibialis anterior muscle).

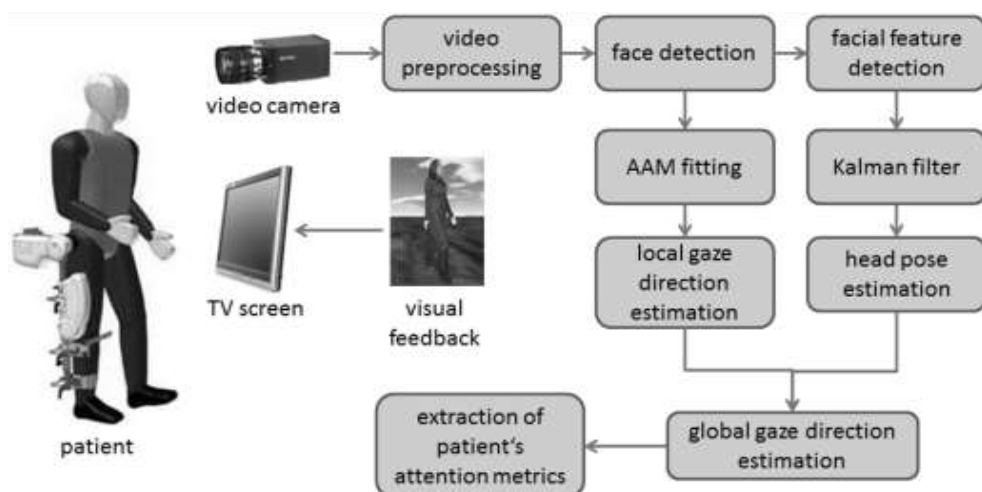


Figure 1. Overview of the proposed approach for video-based estimation of patient's attention/compliance during robot-enhanced rehabilitation.

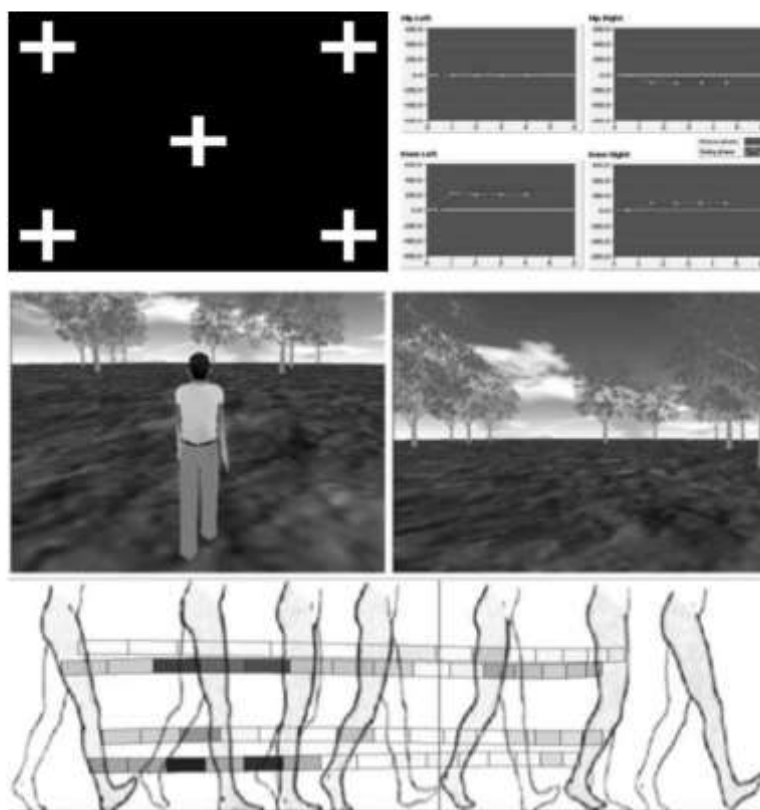


Figure 2. Five different types of visual feedback that are shown to the patient during therapy. Top row: the calibration screen for gaze direction estimation, and gait trainer's plots of current leg positions. Middle row: realistic VR environment from 1st and 3rd person perspective. Bottom row: special feedback displaying muscle activations.

The video streams are processed by our algorithms. First, frontal faces are detected and main facial features are extracted (corners of the eyes, pupil centers, mouth, tip of the nose). To suppress jitter from detection errors and body swings during walking, a Kalman filter is applied to the locations of the extracted facial features. Next, an active appearance model (AAM (11)) of the face with 59 facial landmarks is used to more accurately represent the current facial pose/expression. The relative distances between extracted landmarks are used to calculate the head pose and gaze direction. Finally, different metrics of the patient's attention/compliance are computed: the percentage of time the patient is observing the visual feedback, the spatial gaze distribution maps, the responses to actions in the virtual reality (VR) environment, etc.

We tested five different types of visual feedback (see Figure 2): a calibration screen for the initialization of our algorithms; 2D plots of current leg positions; 2D plots of muscle activations; and two realistic VR environments (walking in a park from 1st and 3rd person perspectives). The VR environments were created in OpenGL 3.3 and consist of ground, sky dome and male/female avatars. The avatars were created in Mixamo Pro Character Creator Tool and animated with Autodesk 3DS Max 2012. Both avatars have an underlying bone structure with all major joints modeled, allowing animation of almost all human movements. The actual avatar movement within the VR environment is controlled by kinematic data from the robotic trainer, so leg movements in VR world correspond with leg movements in real life.

The whole video processing system was designed for real-time operation, but the current version of the software is a mix of C++ and Matlab routines and runs at approximately 1 frame per second. Therefore, all video processing is currently performed offline, but (near) real-time operation is possible with further optimization of the code.

EXPERIMENTAL RESULTS

The proposed system was evaluated in an experiment that involved 4 stroke patients and consisted of 5 runs of robot assisted walking with different forms of visual feedback. In all runs, the patients were instructed to walk actively, maintaining a constant speed, and applying minimum force on the robot. A 42" screen was placed in front of the patient, 1.4 m away from his face. Each run lasted 4 minutes. For all types of visual feedback the walking speed remained constant. During the experiments videos of the patient's face were recorded to disk in HD resolution and later processed offline.

On average, the face was detected in 93% of video frames recorded (i.e., in practically all the frames with frontal faces whereas the profile faces were not detected). Eyes, mouth, nose, and pupils were accurately detected in more than 99% of detected faces (see Table 1). Detection of facial features was skipped in video frames where no face was detected. The average jitter of detected facial features was 1.0 ± 0.4 pixels. The AAM was successfully fitted to all frontal faces. The average jitter of AAM landmarks was estimated at 0.6 ± 0.4 pixels.

Table 1. Facial feature detection performance, estimated on three test videos

	Video 1 (gaze targets)		Video 2 (gaze targets)		Video 3 (VR 3 rd person)	
	frames	%	frames	%	frames	%
Whole video	9999	100 %	13391	100 %	10551	100 %
Face detection	9833	93.7 %	13193	98.5 %	10427	98.8 %
Detection of left eye	9805	99.7 % [*]	13174	99.9 % [*]	10419	99.9 % [*]
Detection of right eye	9769	99.3 % [*]	13179	99.9 % [*]	10408	99.8 % [*]
Detection of left pupil	9800	99.9 % [#]	13191	99.9 % [#]	10419	100 % [#]
Detection of right pupil	9769	100 % [#]	13187	100 % [#]	10408	100 % [#]
Detection of nose	9827	99.9 % [*]	13150	99.8 % [*]	10423	99.9 % [*]
Detection of mouth	9742	99.1 % [*]	13174	100 % [*]	10414	99.8 % [*]

^{*} Values normalized by the number of face detections.

[#] Values normalized by the number of eye detections.

Videos recorded during sessions with the screen displaying calibration targets were inspected by an expert and 9 approximate gaze directions (top-left, top-centre, top-right, bottom-left, bottom-centre, bottom right, left-centre, right-centre and centre of the screen) were manually annotated. The time periods corresponding to eye movements or eye blinks were ignored and were not annotated. Gaze direction was then calculated automatically by our algorithm and compared to the manually annotated gaze directions. The gaze directions were identified with average accuracy of $94\% \pm 6\%$. Most errors originated from distinguishing between top-left vs. bottom-left and top-right vs. bottom-right gaze directions.

Figure 3 shows an example of a patient's detected level of attention to visual feedback, estimated as percentage of time, in 1 second intervals, with gaze fixed to the TV screen; all gazes outside the screen were classified as non-attention.

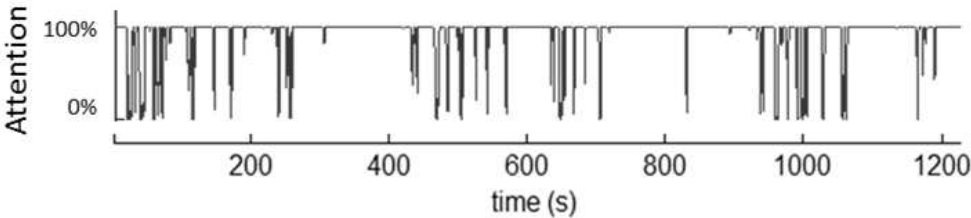


Figure 3. An example of estimated level of a patient's attention to visual feedback during a 20 minutes long rehabilitation session (5×4 minutes).

Figure 4 presents an example of the estimated spatiotemporal gaze distribution metric. This metric shows relative frequency of identified gaze locations over a 4 minute long gait rehabilitation session. Brighter spots in the gaze distribution plots indicate areas with more frequent attention. Such maps support the identification of gaze targets (i.e., gaze hot-spots) and the assessment of spatiotemporal correlation between a patient's attention to visual feedback and other BNCI-based performance indices of gait rehabilitation.

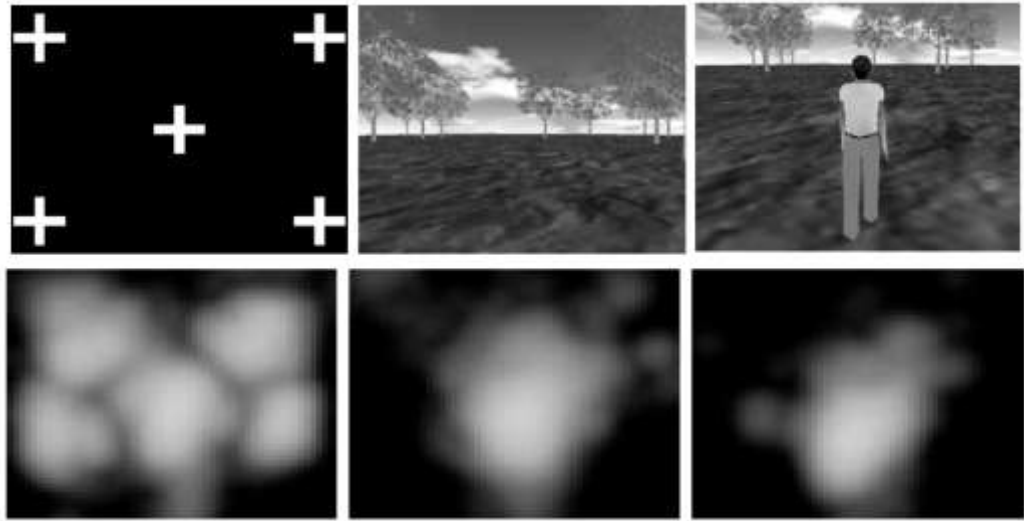


Figure 4. An example of spatial gaze distribution plots (bottom row) for three different types of visual feedback (top row) shown to a patient during 4 minutes long gait rehabilitation sessions.

CONCLUSION

We developed and validated a video-based system for the robust tracking of a patient's face pose and gaze direction during robot-assisted lower limb rehabilitation therapy. The results (see Figures 3 and 4) show that such a system can be used to unobtrusively analyze patient's attention to displayed visual feedback and to provide a means for long-term quantification of visual feedback effects on the rehabilitation progress. It can also serve as an additional feature for other BNCI performance indices, such as similarity of motor modules, kinetic and kinematic profiles and brain patterns. For example, preliminary results show a significant correlation between attention to muscle activation plots and improvements in muscle activations during walking.

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Chapter 86

COLOUR-CHECK IN STROKE-REHABILITATION GAMES

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ABSTRACT

This chapter presents the colorimetric testing of rehabilitation games designed for the StrokeBack project. In this testing, the main subject of the investigation was how the people with different colour-blindness types can perceive the games. Many programmers and game designers do not pay attention to the accessible aspect of games. This accessibility implies that users who are colour-blind should be able to use the games the same way as the people with no vision impairment.

INTRODUCTION

The purpose of the research described here is to investigate the colour palette of virtual reality (VR) based rehabilitation games [1], as designed for the ‘StrokeBack’ [2] project. The goal of the StrokeBack project is to improve the speed and quality of stroke recovery through the development of a telemedicine system which supports ambulant rehabilitation in home settings for stroke patients with minimal human intervention. During our investigation we designed our VR based rehabilitation games in such a way that they would also be enjoyable for people who have colour-blindness.

The disorders in colour vision can be inherited and acquired. The cones’ red and green colour specific paint cell’s genes are linked to the X chromosome. Because of the gender-linked inheritance, this type of impairment is 20 times more prevalent in men. About 8% of

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Caucasian males and 0.4–0.5% of females are “red-green” colour-blind. Inherited blue-blindness (tritanopia) is much rarer – only about 0.05% of the population can be detected. Some colour vision disorders are not inherited, they are so called “acquired”; several ophthalmological diseases can result in colour perception disorders (e.g., retinal diseases, glaucoma, cataracts, etc.) [3].

Stroke is a leading cause of death in the United States, killing nearly 130,000 Americans each year – that is 1 of every 20 deaths [4]. Every year, about 800,000 people in the United States have a stroke. About 610,000 of these are first or new strokes; 185,000 are recurrent strokes. Evidence from opticians indicates that about 10% of the male population are colour-blind. This, together with stroke incidence rate, leads to a statistically important problem.

STATE OF THE ART

The harmony of colours and proportions play an essential role in the appearance. Although many tend to neglect these issues, numerous scientific research studies are concerned with this field. All this is based on the colour perception of the eye, which, however unconsciously, may have an influence on patients’ decisions.

Scientists and artists over the last centuries [5–7] and nowadays [8] developed colour order systems where they defined rules to establish harmonic sets of colours. These colour harmony studies were based on the orderly arrangement of colours in the colour order system. The second group of authors [9, 10] defined colour harmony as an interrelationship of colours. The main principles of these studies are “complementary” and “analogous” but these concepts are not consistent among the studies. Also, the colour wheel was often adopted as a tool to define these basic relationships. Judd and Wyszecki [11] define colour harmony as a more universal concept: “when two or more colours seen in neighbouring areas produce a pleasing effect, they are said to produce colour harmony”. In addition, there is no consistency among the principles and the keywords of colour harmony: it is *completeness* according to Goethe [9], *order* according to Nemcsics [8] and Chevreul [10], and *balance* according to Munsell [6]. A quantitative model for two-colour combinations based on the CIECAM02 colour appearance model was developed by Szabó et al. [12]. Many other effects (i.e., age, cultural background) of colour harmony feeling have yet to be investigated.

In visual experiences, harmony is something that is pleasing to the eye. It engages the viewer and it creates an inner sense of order, a balance in the visual experience. When something is not harmonious, it is chaotic. At one extreme is a visual experience that is so bland that the viewer is not engaged. The human brain will reject under-stimulating information. The human brain rejects what it cannot organize, what it cannot understand. The visual task requires that we present a logical structure. Colour harmony delivers visual interest and a sense of order. Extreme unity leads to under-stimulation, extreme complexity leads to over-stimulation. Harmony is a dynamic equilibrium [13].

Colour deficiency is often neglected, as most people do not consider colour deficiency as a serious problem. Up to 15% of the population being affected by one form or another of colour deficiency [13]. It is quite common to see combinations of background and foreground colours that make web-pages, software, games etc. virtually unreadable for colour deficient users. Background, text, and graphics colours should be carefully chosen to allow

understanding for people with colour deficiency. Designing for colour deficient people is complicated. It is not simply a matter of green/red or yellow/blue combinations [13]. The most important issue in designing for colour deficient users is not to rely on colour alone to convey information and not to use colour as a primary means to impart information [14].

METHODS

Currently 3 games are built in the StrokeBack project:

- Break the Bricks game: practices horizontal (right-left) wrist movements – the player's task is to control the object at the bottom of the monitor;
- Birdie game: practices vertical (up-down) wrist movements – the player's task is to make a little bird fly and not let it fall;
- Gardener game: practices finger extension movements – the player's task is to pump a virtual sprinkler to make the plants grow by watering them.

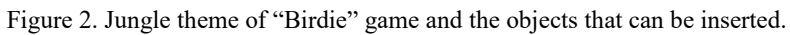
These games can be used during the home rehabilitation process by the patients or replacing/enhancing clinical rehabilitation, thereby speeding up the process of recovery. These games are single player games which the patient can play by himself/herself. It is expected that the patients will play more with the games than they would do exercises. To these games several 'locations' were developed:

- Break the Bricks: default bricks, car, cake, duck, ship, train.
- Birdie: default meadow, cave (played with a bat), circus, forest, jungle, mountain, sea, town, winter.
- Gardener: bluebell, carrot, currant, geranium, grape, pepper, rose, strawberry, tomato, tulip.

Besides the 24 backgrounds for the 24 themes, more than 170 different objects had to be inserted, each of which had to be clearly visible. These were tested by different colour-blindness simulators and automatic testers.



Figure 1. Gardener's "Bluebell" and "Grapes" themes as seen by a person with good vision.



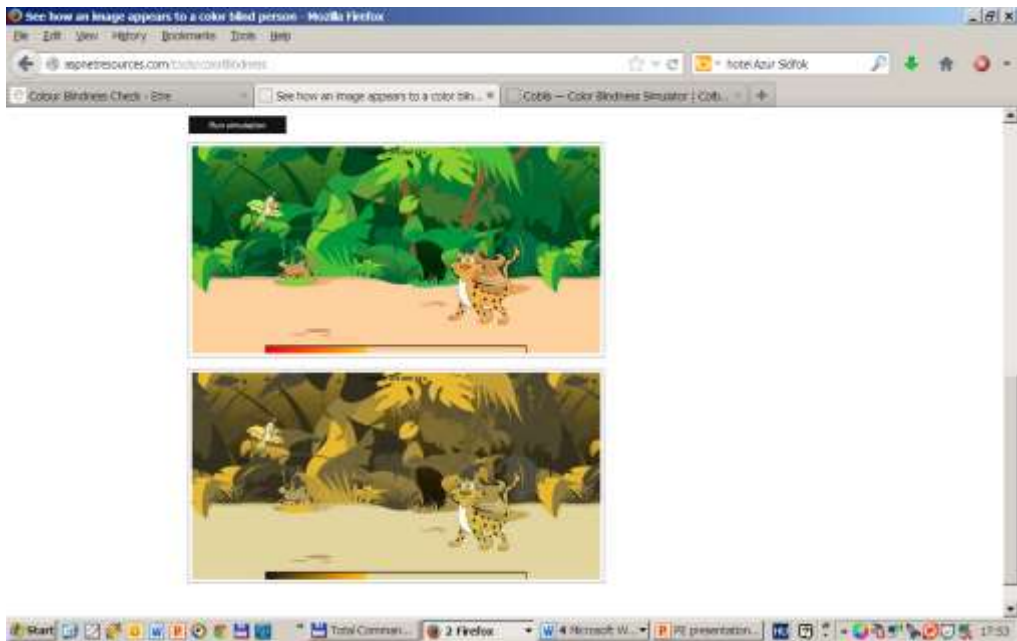


Figure 3. Testing of “BTB basic brick” theme with ETRE simulator (upper) and “Birdie jungle” theme with ASP.NET simulator (lower).

RESEARCH AND DEVELOPMENT

For the investigation four different colour-blindness simulators were used, which are accessible on the Internet and where pictures can be uploaded [15–17], and a downloadable software, ColorOracle [18], with which we could test the pictures appearing on the screen on the developer computer to find out how the users of different types of colour-blindness – deuteranopia, protanopsis, tritanopia – see the colours.

Testing and Contribution to the Field

Testing has been completed not only with the *ETRE*-, *ASP.NET* based-, and *Coblis*-simulators and the *ColorOracle* tester, but also with Variantor’s special glasses [19], with two individuals with good vision, and two persons with colour-blindness. Based on prevalence (10%) of colour-blindness in the male population, and the case that colour-blindness is aggravated by stroke [20], individual tests using a standard colour chart display has been conducted. Figure 5 shows the difference between normal colour vision (Figure 5a) and colour blindness in the red tone (Figure 5b).

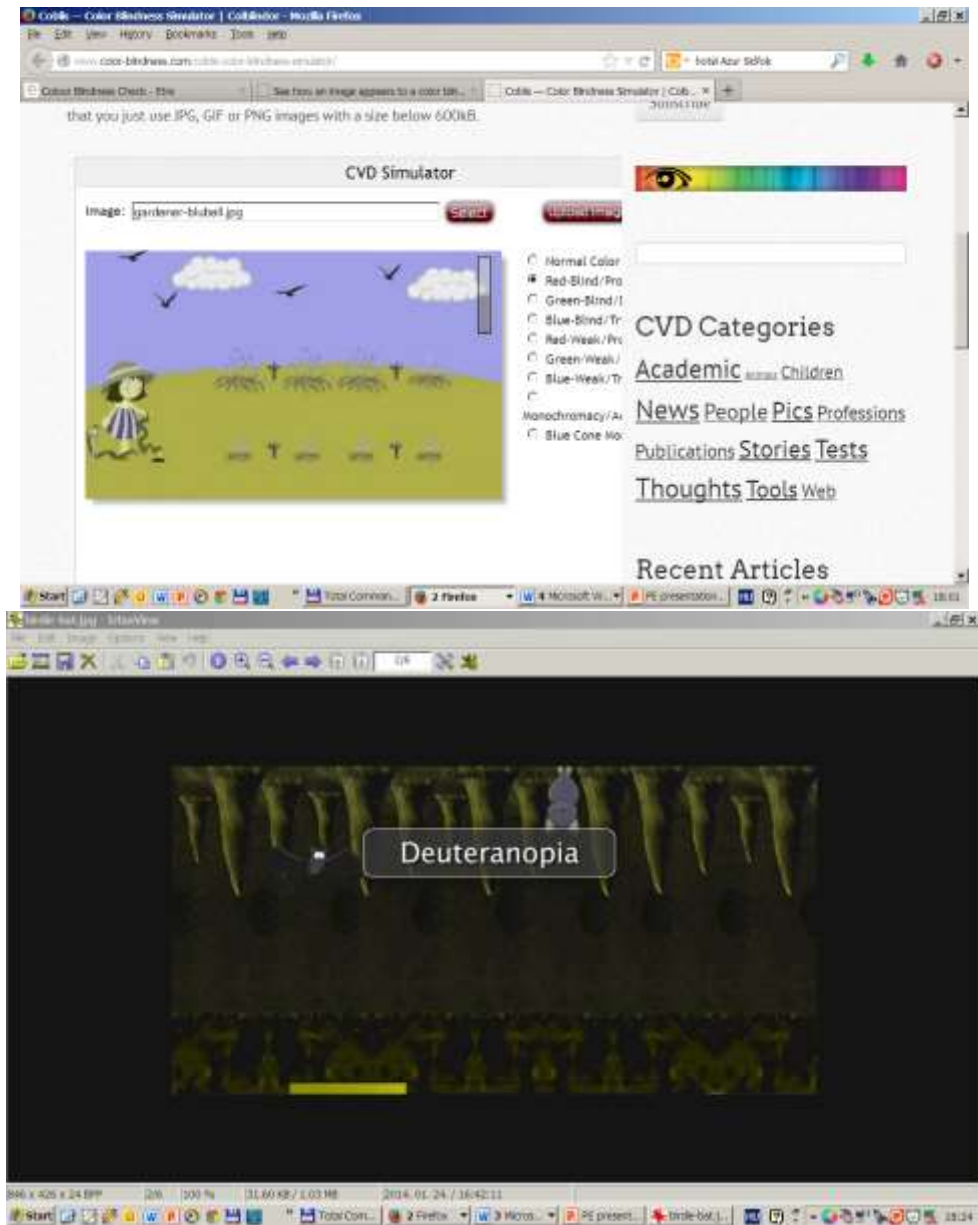


Figure 4. Testing of “Gardener” with Cobli simulator (upper) and “Birdie cave” theme with ColorOracle tester.

The tests were completed by people with normal vision and those with colour-blindness. The visual elements of the games were not just tested with online applications. Out of the possible test subjects, we filtered for colour-blindness by applying the Farnsworth-Munsell 100 HueColor Vision test [21].



Figure 5. Original colour chart (left). Colour chart vision with Variantor glasses (right).

During the test, the subjects had to look at some parts of images taken from the games and were then asked questions regarding them. The questions concentrated on visibility and perceptibility, and the elements disturbing or obstructing visibility and perceptibility. Example questions are:

- *Jungle theme*: What kind of fruits could you see on the tree? How many? Which animal has a darker colour?
- *Cave theme*: How many bats have been in the cave? What objects could you see on the ground beneath the crab?
- *Forest theme*: Was there a wooden high-stand in the picture? What colour are the mushroom caps? How many separate clouds are visible?
- *Winter theme*: How many white animals could you see in the picture? From which direction did the bird (if there has been) fly to the image?
- *General questions*: What value did the achievement bar show on the right hand side?

Based on the test subjects' opinion, the achievement bar has been placed on the periphery of the screen and for the most of the time they have not even noticed that it is placed there. In the test subjects' judgement, the games' visibility and perceptibility is good, although they have indicated that, in the circus theme, the white and red stripes of the circus tent have been disturbing, which generated the vibrating effect. In the case where the recurrence of some image elements have been questioned, the elements have been recognized in the pictures and the subjects have even been able to determine the correct number, but the colours of the elements could not be recalled. As an example, in the garden topic the subjects were able to tell how many tulips have there been on the picture, but they could not tell the colours of the flowers. Responses to questions on colour balance include remarks that the plants in the picture mostly fade into the greenish hue background and, in case of the jungle theme, the green parrot can hardly be noticed between the leaves of the tree.

For some of the users and test subjects the noisiness and pixel problems due to the low image resolution were disturbing. The test subjects have also emphasized some situations that had disconcerting effects, for example when combinations of colours have been shown which physically interrupted the focusing of the eyes, as in the case of combination red-blue colours.

The results of the tests have shown that the observers – despite their colour vision deficiency – have seen picture elements taken from the game appropriately, the small details

having been recognized and identified. The separation, observation and recalling of the games' image elements did not cause any problems for the validly colour-blind test subjects.

CONCLUSION AND PLANNED ACTIVITIES

We have not found any publications that consider the testing of colours of rehabilitation games, lending credence to our view that developers do not think about the colour-blind people. As a result of our testing, we reached the conclusion that, within the StrokeBack games, the objects are clearly visible, so the colour-blind patients can practice in the same way as the normal sighted. In future, games should not only be tested with the software, but also with other simulation goggles (tunnel-vision, macula or lens degeneration). Clinical tests of efficiency (which refer to the colour testing and efficiency of the rehabilitation) were started in June, 2014, and future reporting will detail these results.

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Chapter 87

STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS: INSIGHTS ON NEURONAL VULNERABILITY AND ASTROCYTIC ABNORMALITIES IN STROKE

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ABSTRACT

Stroke evokes cerebral infarction and hemorrhaging and is associated with very high mortality. Stroke-prone spontaneously hypertensive rats (SHRSP) exhibit severe hypertension, and more than 95% of them die of ischemic cerebral stroke. In SHRSP rats, ischemic stresses such as hypoxia and re-oxygenation result in enhanced neuronal cell death *in vivo* and *in vitro*. Specific characteristics of brain cells from SHRSP rats contribute to their susceptibility to stroke. For example, the susceptibility of SHRSP rat neurons to cell death is partly due to an insufficiency of mitochondrial redox regulation and a deficiency of the apoptosis-inhibitory protein Bcl-2. However, the proliferative capacity of astrocytes isolated from SHRSP rats is significantly increased compared with those from the parental Wistar Kyoto Wistar Kyoto strain. In astrocytes, lactate production acts as an energy source for neuronal cells, and is reduced in SHRSP cells compared to normal WKY rats. Furthermore, SHRSP astrocytes show decreased production of neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF) and L-serine when compared to WKY astrocytes after hypoxia and re-oxygenation. After stroke, neuronal survival alone may be insufficient to promote recovery. Thus, in recent years, there has been an elevated focus on the roles of astrocytes in stroke. In this review, we provide insights on neuronal vulnerability and astrocytic abnormalities during stroke.

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Keywords: SHRSP, neuronal cell, apoptosis, astrocyte, GDNF, Bcl2, hypoxia and reoxygenation, lactate, ROS, L-serine, stroke, thioredoxin, VCAM-1, vitamin E, WKY

ABBREVIATIONS

AVP	arginine vasopressin
BBB	blood-brain barrier
CNS	central nervous system
EGF	epidermal growth factor
GDNF	glial cell line-derived neurotrophic factor
HMGB1	high-mobility group box 1
KA	kainic acid
MCAO	middle cerebral artery occlusion
MCP-1	monocyte chemotactic protein-1
NHE	Na/H exchange
NKCC	Na-K-Cl cotransporter
PDGF	platelet-derived growth factor
3-PGDH	3-phosphoglycerate dehydrogenase
ROS	reactive oxygen species
SHRSP	stroke-prone spontaneously hypertensive rats
TGF- α	transforming growth factor- α
Trx	thioredoxin
TNF- α	tumor necrosis factor- α
VCAM-1	vascular cell adhesion molecule-1
WKY	Wister Kyoto

1. INTRODUCTION

Stroke induces cerebral infarction and hemorrhaging and is associated with very high mortality worldwide [1]. At the onset of stroke, brain dysfunction and neuronal loss occur due to an insufficient blood supply to the brain. Stroke-prone spontaneously hypertensive (SHRSP) rats develop severe hypertension, greater than 200 mm Hg, and more than 95% of them die of stroke [2, 3]. In SHRSP rats, enhanced sodium intake accelerates the rise in blood pressure (BP) and cerebral ischemia induces the appearance of cerebral vasogenic edema [4]. Therefore, SHRSP rats are widely used as an experimental model of human stroke [3].

Numerous studies using SHRSP rats have provided valuable information regarding human stroke and have identified genetic susceptibility to particular types of cerebrovascular diseases associated with stroke [5]. For example, twenty minutes of cerebral hypoxia stimulation in SHRSP rats potentiated a large efflux of glutamate, causing significant but delayed neuronal death in region CA1 of the hippocampus, whereas rats from the parental Wister Kyoto (WKY) rat strain did not exhibit this pathology under the same conditions [6]. Thus, it appears that hippocampal neurons of SHRSP rats are innately vulnerable to ischemic stimulation such as hypoxia. However, Ca²⁺ channel blockers were able to prevent neuronal

cell death in SHRSP rats [7]. It was reported that neuronal production of hydroxyl radicals was significantly elevated after reperfusion or hypoxia in SHRSP rats [6]. In contrast, neuronal expression of thioredoxin (Txn) and Bcl2 significantly decreased in SHRSP rats compared with WKY rats. Both *in vitro* and *in vivo* studies have demonstrated that SHRSP rat neurons are more vulnerable than WKY neurons during cerebral ischemia-hypoxia [8, 9]. In these reports, it was suggested that unknown factors other than a genetic weakness in the neurons themselves also played a role in accelerating cell death in SHRSP rats during cerebral ischemia [8, 9]. For example, the astrocytes of SHRSP rats were largely altered by ischemic stimuli such as hypoxia. Lactate production from these astrocytes (which acts as an energy source for neuronal cells) was decreased in SHRSP rat cells compared to WKY astrocytes [10]. In addition to decreased lactate production, during hypoxia and reoxygenation (H/R) SHRSP astrocytes also showed reductions in glial cell line-derived neurotrophic factor (GDNF) and L-serine in comparison to WKY astrocytes. Furthermore, the production of L-serine and the expression of lactate transporter occurred at lower levels in SHRSP astrocytes than in WKY astrocytes after exposure to arginine vasopressin (AVP) [11].

Oxygen reperfusion after cerebral ischemia rapidly produces a large quantity of reactive oxygen species (ROS) and induces neuronal cell death in SHRSP rats. However, the pathogenic mechanisms of stroke in SHRSP rats are not well understood. Cerebral ischemia induces destruction of the blood-brain barrier (BBB), subsequently increasing edema and nervous system cell death. In SHRSP rats, vascular endothelial injury is observed at multiple sites following BBB leakage, ultimately resulting in vessel rupture [12, 13]. After stroke, neuronal survival alone may be insufficient to promote full recovery. Thus, in recent years there has been an elevated focus on the roles of astrocytes in stroke. In this review we present an overview of the cellular characteristics and function of SHRSP and WKY rat neurons and astrocytes during cerebral ischemia and hypoxia.

2. NEURONAL VULNERABILITY AND REDOX REGULATION IN THE BRAIN OF SHRSP RATS UNDER ISCHEMIC CONDITIONS

2.1. Vulnerability of Neuronal Cells in SHRSP Rats

In the SHRSP rat stroke model, ischemic stimulation is considered to be the most effective mechanism for promoting neuronal cell death during cerebral ischemia. *In vivo* studies have shown that a cerebral ischemic event lasting 20 minutes can induce the production of large amounts of glutamate, causing delayed neuronal cell death in the CA1 region of the hippocampus of SHRSP rats [6]. Other studies have also investigated these effects in cultured neuronal cells isolated from fetal brains of SHRSP and WKY rats. The neuronal cells were cultured for 6 to 24 hours under 1% O₂ hypoxic conditions then subsequently exposed for 1.5 to 5 hours to a reoxygenated state to assess cell viability [8]. None of the neuronal cells were stained by trypan blue, indicating the absence of cell death in both strains. Under these conditions, 65%-85% of WKY neuronal cells survived even after 36 hours of hypoxic culture. In contrast, neuronal cells from SHRSP rats showed a marked increase in the percentage of cell death, with almost all of the dead cells being apoptotic [8]. After exposure to hypoxic conditions, with only 1.5 hours of reoxygenation, only 10 to 30%

of neuronal cells survived. The total rate of neuronal cell death in WKY rats and SHRSP rats was 41% and 78%, respectively. The rate of necrosis in neuronal cells of WKY rats and SHRSP rats was 12% and 15%, whereas the rate of apoptosis was 29% and 63%, respectively. Three hours of reoxygenation after hypoxia induced cell death in 68% of WKY neuronal cells, whereas 99% of SHRSP neuronal cells had died. However, little or no DNA fragmentation was observed in neuronal cells of SHRSP rats after culture under normoxic conditions (20% oxygen). After 36 hours of hypoxia and 3 hours of reoxygenation, a marked enhancement in DNA fragmentation was observed, and was generally localized to areas containing many lipid droplets [8]. The apoptotic stages of neuronal cell death were also classified [8, 9]. (A) During the early stage of apoptosis, neuronal axons were present but dendrites were lost and many lipid droplets could be observed in the neuronal cell bodies. (B) During the next stage of apoptosis, cell shrinkage was observed. (C) This was followed by continued development of apoptotic cells. (D) During the final stage of apoptosis, both the neuronal cell membranes and nuclei disappeared.

2.2. ROS Production and Neuronal Injury

Cerebral ischemia induces the rapid generation of a large amount of reactive oxygen species (ROS) and induces neuronal cell damage. This is particularly evident in SHRSP rats in which oxidative stress induces neuronal cell death and redox changes. For example, SHRSP rats generate a large amount of ROS that leads directly to neuronal death after ischemic stimulation such as the reoxygenation that occurs after hypoxia [14]. Cerebral ischemia increases the intracellular level of calcium ions and activates calcium-dependent proteases. Moreover, these changes activate xanthine dehydrogenase (XDH) and xanthine oxidase (XOD). In SHRSP rats, the XOD inhibitors allopurinol or superoxide dismutase prevented apoptosis of neuronal cells during hypoxia and oxygen reperfusion [8]. Similar changes occur upon reperfusion of the ischemic rat heart in which activation of XOD induces ischemic reperfusion injury in cardiomyocytes [15]. Here, XOD is a significant source of free radicals and induces oxidative injury. In cerebellar granule cells, glutamate triggers a large influx of Ca^{2+} which induces the rapid conversion of XDH into XOD and production of ROS [16]. Thus free radicals are produced by reoxygenation after cerebral ischemia and enhance injury of neuronal brain cells [17]. Free radical production is one of the initial steps in a series of events leading to neuronal degeneration and death. The free radical-generating enzyme XOD may be a central regulator of postischemic neuronal cell injury. Alternatively, it has been reported in rats that stimulation by middle cerebral artery occlusion (MCAO) can induce brain damage, massive neuronal loss, and DNA fragmentation. These MCAO experiments demonstrated that endoplasmic reticulum stress enhances brain damage after cerebral ischemia and reperfusion in rats [18].

2.3. Redox Regulation in SHRSP Rats

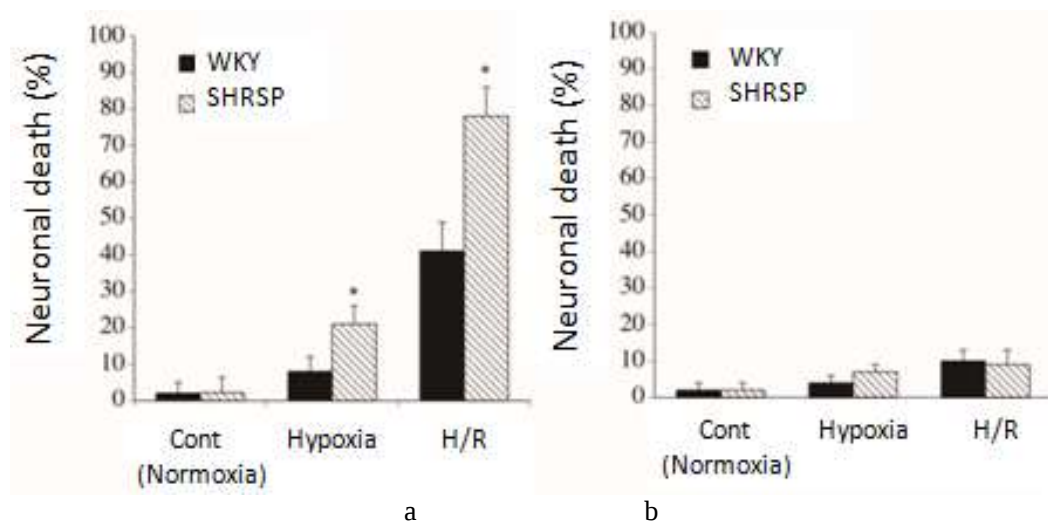
Activation of antioxidants and enzymatic antioxidant pathways reduces ROS formation such as the hydroxyl radicals produced during H/R and is beneficial for preventing neuronal injury [19] (Pan et al., 2015). This neuronal protection might be achieved by increasing the

levels of antioxidant substances such as vitamin E [20]. Namely, for the prevention of neurotoxicity by cerebral ischemia, redox regulation through increased levels of antioxidants and antioxidant enzymes is important. Moreover, decreased glutathione (GSH) levels in neuronal cells of SHRSP rats have been associated with neuronal vulnerability [21]. To further investigate this, the redox status of tissues from WKY, SHR and SHRSP rats were investigated. The SHR rat strain showed enhanced oxidative stress and impaired thioredoxin (Trx) expression. Induction of Trx was impaired after treatment with angiotension II in blood mononuclear cells isolated from SHR or SHRSP rats compared with those isolated from WKY rats [22]. The expression of Trx mRNA was detected prior to hypoxia, however, following hypoxia and after reoxygenation Trx expression was significantly reduced in neurons of SHRSP rats compared to WKY rats [23]. Recent studies have demonstrated that Trx has a protective effect on neurons and is closely related to oxidative stress and apoptosis in cerebral ischemic injury [24]. Experiments were conducted to validate the neuroprotective effect of recombinant human Trx-1 and its potential mechanisms against ischemic damage after MCAO in mice. Trx-1 significantly improved neurological function and decreased cerebral infarction and apoptotic cell death 24 hours after MCAO. In addition, Trx-1 resulted in a significant decrease in heme oxygenase-1 (HO-1) which is protective against oxidative stress. These results indicated that Trx-1 exerts a neuroprotective effect on cerebral ischemic injury. Trx-1 may represent a therapeutic approach for reducing oxidative stress-induced neuronal apoptotic cell death in acute ischemic stroke. Trx proteins act against ROS via the sulfhydryl (-SH) group. In addition to ROS antagonism, Trx proteins have many functions pertaining to intracellular signal transduction. Thus, reduced expression of Trx is associated with an attenuation of the defense system of antioxidants during oxidative stress in SHRSP rats. This subsequently results in reperfusion-induced neuronal cell death. These findings suggested that redox regulatory functions in SHRSP rat neurons were markedly inhibited by oxidative stress stimulation after hypoxia, and such changes may be involved in neuronal vulnerability [25].

2.4. Preventive Effects of Vitamin E

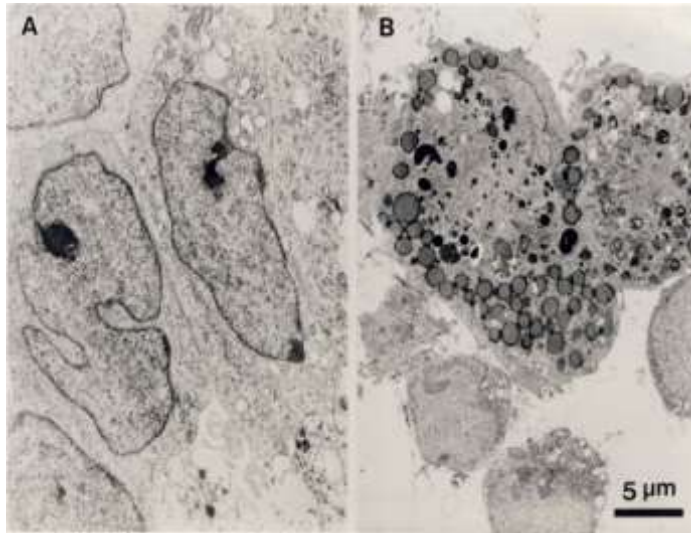
Vitamin E is a powerful antioxidant and its effects on reduced oxidative stress in ischemic stroke have been demonstrated [26]. Specifically, vitamin E, which is present in biological membranes, contains a hydroxyl group that reacts with unpaired electrons and can be reduced to form peroxy radicals. The relationship between vitamin E and reactive oxygen species has been demonstrated in mouse models of vitamin E deficiency. These results showed decreased superoxide production during alpha-tocopherol deficiency [27]. The major antioxidant effect of vitamin E is to quickly add alkoxy radicals and hydrogen to peroxy radicals. Thus, the mechanism of vitamin E is to act as a chain-breaking antioxidant that blocks reactive oxygen metabolic cascades [28, 29]. Vitamin E induces SOD, GST and catalase, and also enhances the levels of GSH and reduces lipid peroxidation. The effects of vitamin E were shown to be more effective than those of vitamins A or C [30]. In WKY and SHRSP rat strains, vitamin E prevented neuronal cell death associated with cerebral ischemia-reperfusion, particularly apoptosis [8, 20]. The overproduction of oxygen-free radicals that occurs in the tissues of SHRSP rats is associated with reoxygenation injury after hypoxia. The effects of vitamin E were also investigated during hypoxia and oxygen reperfusion using

cortical neurons isolated from WKY and SHRSP fetal rat brains at 15 days of gestation. Hypoxia increased the rate of neuronal cell death in SHRSP rats, but did not increase cell death in WKY rats [8, 21] (Figure 1). The percentage of neuronal cell death that occurred mainly via apoptosis during hypoxia and reoxygenation was remarkably higher in neurons from SHRSP rats than from WKY rats. However, in SHRSP rats, neurons incubated with 50 to 300 $\mu\text{g/mL}$ of vitamin E remained intact [8, 21] (Figure 2). Using HPLC, the accumulation of vitamin E was confirmed within the mitochondria of neuronal cells after 36 hours of hypoxia. Vitamin E (when dosed at 50 $\mu\text{g/mL}$) accumulated most effectively into the mitochondria of neuronal cells in SHRSP rats. The presence of vitamin E is most enriched in the microsomal cell membranes of mitochondria of the liver and heart. Transport of vitamin E to the mitochondria or microsomes is achieved by vitamin E binding to the alpha-tocopherol transfer protein (TTP) [31, 32]. Genetic variation in the TTP gene can induce ataxia with vitamin E deficiency. Thus, vitamin E might have significant inhibitory effects against neuronal cell injury after incorporation into biological membranes, as observed in the mitochondrial membranes of SHRSP rats.



In neurons in culture matured, they were incubated in 1% O_2 for 36 hours without (A) or with (B) vitamin E (100 μM). After the hypoxic culture, they were maintained in 21% O_2 for 1.5 hours with or without vitamin E (100 μM). Vitamin E treatment was carried out as described in a previous report (Tagami et al., 1998). Cont (normoxia); 21% for 36 hours, Hypoxia; 1% O_2 for 36 hours. H/R; 1% O_2 for 36 hours plus 21% O_2 for 1.5 hours. Asterisk (*); $p < 0.001$ compared with WKY rats. Values are the mean $\pm$ SE ($n = 7$). Citation from Tagami et al., (1998) and Yamagata et al., (2009).

Figure 1. Inhibition of cell death in cortical neuronal cells by vitamin E during hypoxia and reoxygenation in WKY and SHRSP rats.



In neuronal cells in culture, they were incubated in 1% O₂ for 36 hours with (A) or without (B) vitamin E (100 μM). Vitamin E treatment was carried out as described in a previous report (Tagami et al., 1998). Citation from Tagami et al., (1998) and Yamagata et al., (2009).

Figure 2. Preventive effects on ischemic neural cell death of vitamin E on neurons of SHRSP rats.

2.5. Apoptosis and Neuronal Cell Death in SHRSP Rats

The mechanism of neuronal cell death in SHRSP rats is likely apoptosis [8, 21]. The expression of apoptosis-inducing molecules such as Bax was increased in injured neuronal cells. Conversely, the expression of apoptosis-inhibiting molecules, such as Bcl-2 and Bcl-XL, increased in the surviving neurons of rat brains following global ischemia [33]. Distinct expression of some members of the bcl-2 gene family may play an important role in determining the sensitivity of neurons to ischemic cell death [34]. Expression of bcl-2 after hypoxia and reoxygenation was examined by real-time quantitative-PCR using cultured neuronal cells isolated from SHRSP and WKY rats [23, 35]. In SHRSP rat neurons, apoptosis-inducing molecules such as Bax were increased after damage. The pattern of bcl-2 gene expression in SHRSP and WKY rats showed the most marked difference after 30 minutes of reoxygenation in which bcl-2 was significantly reduced in SHRSP compared to WKY rats [20] (Figure 3). Thus, the sensitivity of neuronal cells to ischemic stress likely accelerates neuronal cell death during stroke in SHRSP rats.

3. CHARACTERISTICS OF SHRSP RAT ASTROCYTES AND CORRELATIONS WITH STROKE

3.1. Roles of Astrocytes in Stroke

Neuronal survival alone may be insufficient to promote recovery after stroke. Thus, in recent years there has been an elevated focus on the roles of astrocytes in stroke. Astrocytes

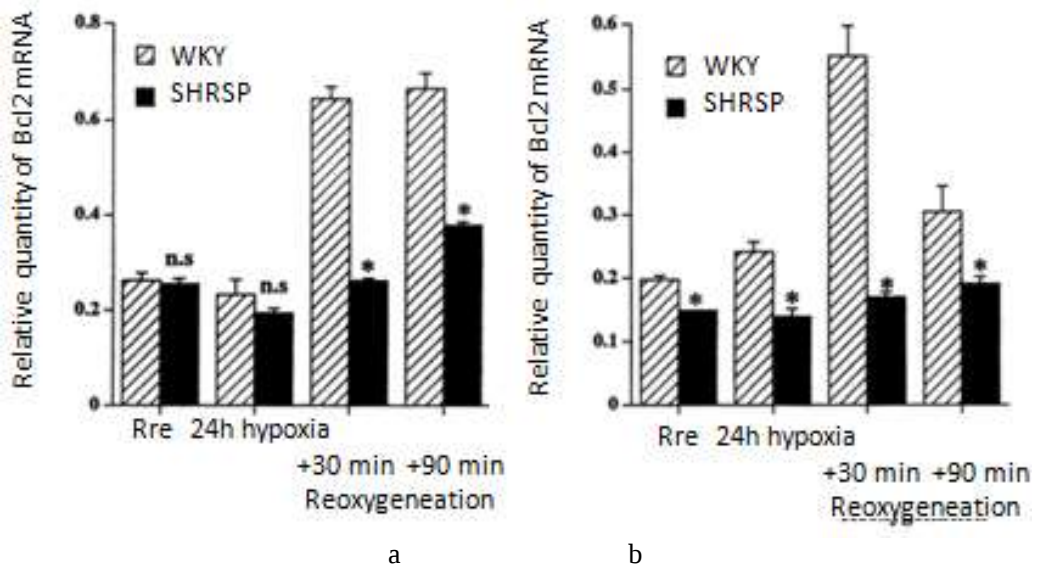
play a number of essential roles in a properly functioning central nervous system (CNS). In particular, astrocytes are important for regulating changes in vascular tone in response to neuronal activity. For example, astrocytes promote the formation and integrity of the BBB, as well as promote synaptogenesis and respond to the production of inflammatory molecules [36]. In stroke, the multiple functions of astrocytic activity are affected. After ischemic injury, reactive gliosis has been observed in the brain of humans and in animal models of stroke [37]. Previous studies have suggested that the phenotypic conversion of astrocytes from the resting to reactive state may be associated with many brain diseases [38]. Severe brain injury results in the proliferation and migration of reactive astrocytes; specific key molecules play a role in regulating the pathophysiological functions of reactive astrocytes [39].

In SHRSP rats, enhanced BP destroys pericytes and promotes the permeability of endothelial cells, causing astrocytic swelling around the blood vessels [40]. Surprisingly, swelling of the astrocytes occurs before neuronal cell death in SHRSP rats [40]. Thus, the astrocytic swelling observed in SHRSP rats may promote neuronal death during ischemic stroke [41]. In cultured astrocytes isolated from SHRSP rat brains, the cell growth rate in 10% FBS was faster than in astrocytes from WKY rats. Specifically, the doubling time of cultured astrocytes isolated from SHRSP rats was 21 hours, whereas that of WKY rat astrocytes was 30 hours. These data demonstrate the elevated growth capacity of astrocytes from SHRSP rats, and suggest that the large number of reactive astrocytes in SHRSP rats may be detrimental. Furthermore, SHRSP rats showed enhanced astrocytic reactivity in response to epidermal growth factor (EGF) compared to astrocytes from WKY rats, and this was associated with increased cell number. These proliferative astrocytes were observed around infarcted tissue in ischemic brains and were immunoreactive for the EGF receptor (EGFR) [42]. Another report demonstrated that transforming growth factor- α (TGF- α) is a ligand for the EGF receptor (EGFR), and can prevent ischemic neuronal damage. The enhanced proliferative activity of SHRSP rat astrocytes suggests that neuronal damage in cerebral ischemia may be due to an excessive response to EGF [43]. Furthermore, it has been reported that ischemic insult enhanced the expression of platelet-derived growth factor (PDGF) β -receptor mRNA and protein levels in the brain of SHR rats [44]. Thus, the enhanced proliferative activity of astrocytes from SHRSP strain rats suggests that cerebral vascular lesions in cerebral ischemia may be due to dysfunctional responses to EGF and PDGF [42, 44].

3.2. GDNF Production in Astrocytes and in SHRSP Rats

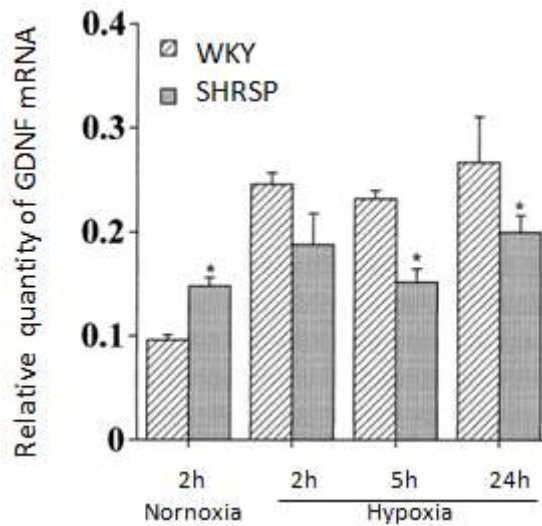
Astrocytes regulate the uptake of glutamate [45], water and ion homeostasis [46], the induction of the blood-brain barrier (BBB) [47], as well as induce production of cytokines [48] and neurotrophic factors [49]. For example, after brain injury, neuronal cell regeneration is regulated through the release of neurotrophic factors from astrocytes [49, 50]. In addition, GDNF is released after ischemia and has a protective effect against ischemic brain injury [51]. The release of GDNF was shown to be enhanced after transient MCAO in the cerebral cortex [52] or after kainic acid injection [53]. Moreover, the expression of GDNF and its receptor are induced in brain tissue under post-ischemic reoxygenation conditions [54]. Previous studies have demonstrated that the vulnerability of SHRSP neurons to cell death under ischemic conditions such as hypoxia and reoxygenation was associated with decreased

GDNF production by SHRSP astrocytes [55]. Cultured astrocytes isolated from SHRSP rats released higher levels of GDNF than did WKY rats [23] (Figure 4). This suggests that during injury of the CNS in SHRSP rats, the attenuated release of GDNF by astrocytes may be associated with neuronal vulnerability. Furthermore, after 1% O₂ hypoxia and 1.5 or 6 hours of reoxygenation, the release of GDNF was significantly lower in astrocytes from SHRSP strain rats than WKY rats. Similarly, the addition of sphingosine 1-phosphate (S1P) [56] and adenosine [57] partially blocked the release of GDNF from cultured astrocytes isolated from SHRSP rats. S1P is a lysophospholipid secreted by activated platelets [58], and induces apoptosis in neuronal cells of the CNS [59]. Under normal conditions, increased S1P attenuates GDNF release by astrocytes and reduces neuronal injury via the production of neurotrophic factors. However, the level of GDNF released by adenosine-exposed astrocytes was significantly reduced in SHRSP rats compared with WKY rats. In the CNS, adenosine has immediate effects, such as in neurotransmission, and longer term neurotrophic effects that promote changes in cell metabolism and structure, in addition to neuroprotective functions [60, 61]. Adenosine increases the level of several growth factors in ischemic brain tissues, likely as part of a preventive response. Furthermore, within the extracellular space, adenosine acts as an intercellular signaling molecule, however, at high levels it induces apoptosis in the CNS [62]. These results indicate that the release of GDNF is regulated by S1P and adenosine during reoxygenation and ischemia. Although the pathogenesis of GDNF release by SHRSP rats is unknown, the metabolic properties of SHRSP rats may be related to the increased expression of GDNF.



Total cellular RNA was isolated from cultured cortical neurons at the following four time points; untreated (Pre), 24 hours of hypoxia (1% O₂), 24 hours of hypoxia, and 30 and 90 minute of reoxygenation (20% O₂). Total RNA was transcribed with reverse transcriptase and amplified by real-time quantitative PCR. These results represent three independent experiments. Bars are the mean $\pm$ SD (n = 4). *P, 0:01 compared with WKY (control) neurons (oblique line column); n.s., not significant. Modified from Yamagata et al., (2000).

Figure 3. Expression of Bcl-2 and TRX-1 mRNA during hypoxia and reoxygenation of cultured neuronal cells isolated from WKY and SHRSP rats.



Confluent astrocytes were exposed to normoxia (5% CO₂ and 95% air) for 2 hours in a sealed chamber and then to hypoxia (1% O₂, 5% CO₂, and 94% N₂) for 2, 5, and 24 hours. Total cellular RNA was isolated from cultured astrocytes. Total RNA was transcribed with reverse transcriptase and amplified by real-time quantitative polymerase chain reaction (PCR). Bars are the mean $\pm$ SD (n = 5). *P < 0.05 compared with WKY (control) astrocytes. Citation from Yamagata et al., (2002).

Figure 4. Regulation of glial cell line derived neurotrophic factor (GDNF) mRNA expression during hypoxia and reoxygenation in astrocytes isolated from SHRSP rats.

3.3. Regulation of L-Serine Production and Neuronal Cell Survival

L-serine plays an important role in neuronal survival by evoking a variety of biological responses within astrocytes. In the brain, L-serine is synthesized by 3-phosphoglycerate dehydrogenase (3-PGDH) in astrocytes but not in neuronal cells [63]. L-serine produced by astrocytes is transported to the extracellular space by neural amino acid transporter ASCT1 and becomes available to neuronal cells where it promotes neurite outgrowth from ganglion neurons and enhances neuronal cell survival [64, 65]. The production of L-serine is induced in response to glutamate, kainic acid (KA) and free radicals in excitotoxic lesions which promote degenerative diseases in the CNS [66]. Furthermore, excitotoxic damage induces the expression of 3-PGDH and ASCT1 proteins in the hippocampus of the mouse brain [66]. A previous report has characterized the altered regulation of L-serine in astrocytes of SHRSP rats. In cultured astrocytes isolated from SHRSP rats, the glutamate-enhanced stimulation of L-serine production was reduced [35, 23] (Figure 5). Moreover, the level of L-serine and expression of PHGDH and ASCT1 was decreased in astrocytes of SHRSP rats compared with WKY rats after glutamate exposure. Thus, reduction in L-serine release from astrocytes may be associated with the neuronal vulnerability to injury in SHRSP rats during stroke. During cerebral ischemia, astrocytic swelling induces ischemic neuronal cell death. Arginine vasopressin (AVP) and hypoxia can both contribute to ischemia-induced astrocytic swelling such as edema [67]. Additionally, AVP also induces inflammatory molecules in traumatic neuronal injury [68]. Ischemic conditions such as hypoxia and stimulation of AVP can affect cerebral cell volume [69, 70], ion uptake by cerebral cells via Na/H exchange (NHE) [71] and

activity of the Na-K-Cl cotransporter (NKCC) [72, 73]. Combined, these results suggest that AVP might contribute to astrocytic swelling induced by hypoxia and reperfusion in SHRSP rats. L-serine released by astrocytes has been shown to support neuronal cell survival. For example, L-serine synthesis and the expression of ASCT1 were shown to be essential for neuronal survival and differentiation [74]. As described above, the expression of 3-PGDH and ASCT1 proteins is induced by excitotoxic lesions in the mouse hippocampus [66]. Moreover, AVP enhanced the production of L-serine and the expression of neural amino acid transporter ASCT1 in WKY/Izm and SHRSP/Izm astrocytes [11]. The production of L-serine and the expression of ASCT1 showed greater attenuation in SHRSP/Izm than in WKY/Izm astrocytes [11] (Figure 6). The AVP-induced decrease in ASCT1 expression in astrocytes is associated with lowered L-serine production in SHRSP/Izm astrocytes. Therefore, L-serine production might be regulated by astrocytes in response to a variety of molecules such as AVP that enhance brain edema in SHRSP rats.

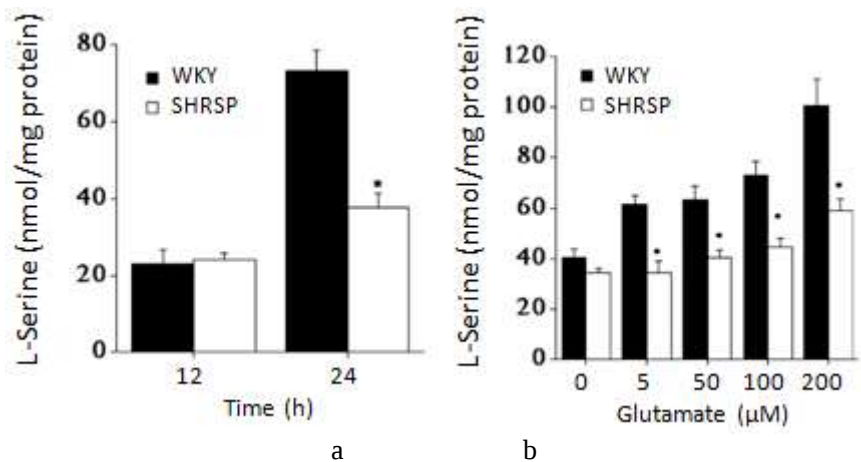
3.4. Regulation of Inflammation by HMGB1 in SHRSP Astrocytes

The High-mobility group box 1 (HMGB1) protein has been reported to be involved in inflammatory responses associated with stroke after ischemia [75]. HMGB1 controls nucleosomal structure stabilization, and regulates inflammation during recovery after stroke [76, 77, 78]. Hypoxia has been shown to enhance HMGB1 expression in neurons and astrocytes [79]. Moreover, after hypoxia, HMGB1 induces the breakdown of the BBB during ischemic insult [80]. HMGB1 was shown to be produced by neurons and astrocytes and exacerbates ischemic neurodegeneration [79]. Production of HMGB1 also promotes endothelial progenitor cell-mediated neurovascular remodeling during stroke recovery in reactive astrocytes [81]. Moreover, during oxygen glucose deprivation and reperfusion, astrocytic HMGB1 release enhances endogenous neural stem cell and/or progenitor cell proliferation [82]. These results suggest that HMGB1 may play an important role in brain tissue repair after an ischemic event [82]. An early study examined the expression of AVP-induced HMGB1 in cultured primary astrocytes isolated from WKY, SHR, SHRSP and SHRpch1_18 rats. AVP induced HMGB1 expression at 50 and 100 nM and was significantly higher in SHR, SHRSP, and SHRpch1_18 rat astrocytes than in WKY astrocytes. The data suggest that HMGB1 may be associated with the early stages of the inflammatory response [80]. This property of SHRSP, SHR and SHRpch1_18 rats is likely an important contributor to enhanced astrocytic inflammation and could explain how AVP augments the inflammatory reaction and promotes neuronal cell death.

3.5. Stimulation of TNF- α and Expression of Vascular Cell Adhesion Molecule-1 in Astrocytes

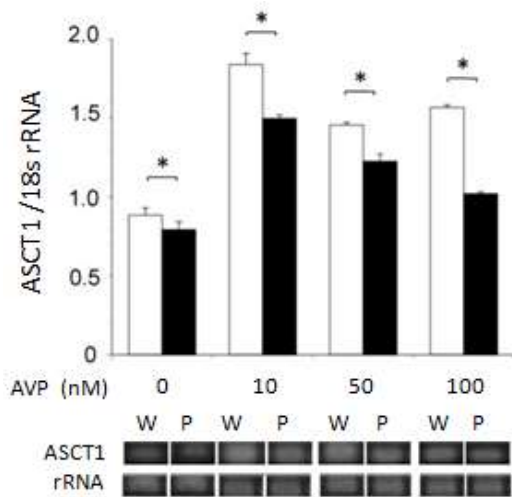
Following exposure to tumor necrosis factor- α (TNF- α), the expression of vascular cell adhesion molecule-1 (VCAM-1) in SHRSP rat astrocytes was enhanced compared to that in WKY rats. The expression of TNF- α and adhesion molecules is related to early neurological exacerbation and infarct volume during stroke [83, 84]. TNF- α is produced by microglial cells and infiltrating macrophages following ischemic stroke [85]. In astrocytes of

SHRSP rats, the expression of monocyte chemotactic protein-1 (MCP-1) was enhanced compared with to normoxic conditions in H/R. The suppression or genetic lack of these adhesion molecules has been reported to decrease infarct volume and edema, and/or mortality in different animal models of ischemic stroke [86]. Enhanced levels of adhesion molecules or TNF- α may be induced by stroke in SHRSP rats during H/R. Thus, the alteration of astrocytic function promotes neuronal cell death during stroke in SHRSP rats.



The confluent growth of astrocytes was arrested for 1 day in FBS-free DMEM containing 0.2% FBS. (a) Glutamate (100 μ M) was added or the cultured astrocytes WKY and SHRSP. Citation from Yamagata et al., (2006).

Figure 5. Effect of glutamate on the secretion of l-Ser in cultured astrocytes isolated from WKY and SHRSP rats.



Confluent WKY or SHRSP astrocytes were exposed to zero, 10, 50 or 100 nM AVP for 8 hours, after which total cellular RNA was isolated for analysis by RT-PCR. Brackets show comparisons of WKY/Izm vs. SHRSP/Izm astrocytes at the same AVP dose. Columns show means $\pm$ SE (n = 4). AVP, arginine vasopressin; W, WKY; P, SHRSP, rRNA, 18S ribosomal RNA. *P < 0.05 compared with WKY (control) astrocytes. Citation from Yamagata et al., (2014).

Figure 6. Arginie vasopressin induced ASCT1 gene expression in WKY and SHRSP astrocytes.

CONCLUSION

The degree of neuronal cell death in the SHRSP rat strain is markedly higher than that in the WKY strain [8, 9]. The properties of SHRSP neuronal cells, unlike those of WKY rats, might be an important factor in the elevated frequency of stroke in cerebral ischemia. In SHRSP rats, vitamin E reduces neuronal cell damage caused by ROS generated after cerebral ischemia. Thus, antioxidants such as vitamin E could be used as a treatment for oxidative stress-mediated neuronal diseases. Vitamin E and other antioxidants may regulate redox potential and apoptosis-related proteins. Furthermore, in SHRSP rats, astrocytic properties contribute to the development of brain disorders. In SHRSP rats, neuronal vulnerability and reduction of astrocytic neurotrophic factors, such as GDNF and L-serine, were demonstrated under cerebral ischemic stroke conditions.

Furthermore, after ischemic stimulation, the production of MCP-1 is strongly enhanced in astrocytes of SHRSP rats [87]. The expression of VCAM-1, MCP-1 and HMGB1 is also markedly elevated in astrocytes of SHRSP rats compared with WKY rats [17]. Of particular note, HMGB1 is known to induce inflammation in brain cells. In summary, neuronal cell vulnerability and abnormal regulation of the supportive functions of astrocytes may elevate the risk of stroke in SHRSP rats.

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Chapter 88

PURE MOTOR MONOPARESIS DUE TO ISCHEMIC STROKE

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ABSTRACT

Pure motor monoparesis (PMM), an isolated paresis due to ischemic stroke in a single arm or leg without higher, cranial, or sensory dysfunction, is an uncommon clinical condition. Cortical infarctions of the precentral knob and anterior cerebral artery territory are the most commonly reported PMM lesions affecting the upper and lower extremities, respectively. Other reported lesion sites include the subcortex, corona radiata, internal capsule, and brainstem; however, they are reported less frequently than cortical infarctions because the descending motor axons in these areas are highly compacted. Large artery atherosclerosis and cardiogenic embolism are considered as the two main etiologies of cortical infarction. In earlier studies, PMM cases due to lacunar infarctions were presumed to be rare. However, lacunar infarction PMM cases have been increasingly reported in the recent years. The weakness patterns in PMM are complex and include proportional and distal- or proximal-dominant weakness. Distal-dominant weakness is the most common PMM type. Some patients exhibit isolated finger paresis or predominant weakness of specific fingers. Although the overall prognosis is favorable in PMM due to ischemic stroke, short- and long-term prognosis depends on risk factors and stroke mechanisms. The diagnosis is sometimes difficult because many cases of PMM lack pyramidal signs and mimic peripheral nerve damage. Therefore, acute monoparesis, particularly in elderly patients with conventional risk factors, should be carefully assessed. Diffusion-weighted imaging is the most useful diagnostic tool for PMM due to ischemic stroke as small cortical infarctions often cannot be detected by conventional sequences of magnetic resonance imaging, or computed tomography. This review provides an update on clinical features, lesion topography, and prognosis of PMM due to ischemic stroke.

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Keywords: pure motor monoparesis, cerebral infarction, diffusion weighted magnetic resonance imaging

INTRODUCTION

Pure motor monoparesis (PMM) of a single arm or leg due to ischemic stroke (IS) without higher, cranial, or sensory symptoms/disturbances is a rare condition [1, 2]; however, it is a clinically important stroke syndrome that can easily be misdiagnosed and/or confused with nonvascular causes of weakness [3, 4]. Undoubtedly, correct diagnosis of acute stroke is of paramount importance to clinicians to enable selection of appropriate treatments and to ensure prevention of acute complications, including recurrent stroke [5]. Given the rarity of PMM due to IS, which often mimics peripheral neuropathy and is sometimes difficult to detect by computed tomography (CT) or conventional magnetic resonance imaging (MRI), many patients with PMM due to IS might have been overlooked until recently. The diagnosis of PMM due to IS has always been a clinical challenge not only for general practitioners but also for neurologists. This review provides an update on the frequency, lesion topography, clinical features, mechanisms, and prognosis of PMM due to IS. Moreover, this review aims to aid clinicians in improving the accurate diagnosis of PMM due to IS, with a focus on differential diagnosis from nonvascular causes of PMM, especially from peripheral nerve damage.

A BRIEF HISTORY OF PMM DUE TO IS

Lhermitte described “pseudoperipheral palsy” in 1909 in patients with a sensorimotor deficit in fingers due to central nervous system lesions [6]. In 1986, Ashizawa et al. reported one patient with ipsilateral leg monoparesis and four patients with ipsilateral arm monoparesis due to brain tumors or brain abscesses located in the contralateral superficial cerebral hemisphere [7], suggesting that monoparesis of the arm or leg due to brain damage was caused by a space-occupying lesion. Between the 1980s and early 1990s, several reports showed superficial infarctions presenting as PMM [8-13]. In 1997, based on a functional MRI study, cortical representation of the hand was determined to be located in the knob-like structure within the precentral gyrus (motor cortex), which was termed the precentral knob; that report also presented a case with hand monoparesis and sensory disturbance due to a precentral knob infarction [14]. Subsequently, numerous studies of hand PMM or monoparesis with sensory disturbances due to precentral knob infarctions were reported. Moreover, diffusion-weighted imaging (DWI), which provides accurate lesion location, led to more frequent reporting of PMM due to IS. In 2005, two large studies from Italy and Switzerland reported the frequency and lesion topography of PMM due to stroke [1, 2]. In 2011, the first English review of PMM due to IS was published from Japan [4].

PMM CRITERIA

Two large studies [1, 2] of PMM due to acute stroke outlined the criteria for PMM as follows: (1) first acute stroke (ischemic or hemorrhagic), (2) a motor deficit in a single body part (face, arm, or leg), (3) no sensory deficit (by subjective complaints and clinical examination), and (4) no visual field deficit. The criteria established by the second study are: (1) limb paresis without any sensory disturbance, coordination or language deficit, or significant involvement of speech or ipsilateral face, and (2) motor deficit caused by ischemic or hemorrhagic stroke, confirmed both clinically and radiologically. The criteria for distal arm PMM due to IS are isolated weakness of one arm or hand that is not associated with objective sensory, coordination, or language deficits, and no significant dysarthria or ipsilateral face or leg weakness [15]. Based on these criteria, PMM due to IS is defined as the presence of an isolated motor deficit in a single leg or arm with no sensory symptoms/disturbances or coordination deficits and no significant involvement of speech or ipsilateral face; the presence of a motor deficit caused by IS is confirmed both clinically and radiologically [4]. Brachiofacial motor stroke [16, 17] is excluded due to facial involvement. A number of previously reported cases of monoparesis due to IS had sensory disturbances, thus they do not fulfill the abovementioned current criteria for PMM due to IS. However, some reports of monoparesis due to IS, especially those with cortical infarctions, include both PMM and monoparesis with sensory symptoms and are often difficult to clearly distinguish. Some of these cases are described below.

FREQUENCY

The reported frequency of PMM due to IS is summarized in Table 1. Earlier studies using CT imaging did not always detect causative PMM lesions; thus, the exact frequency of PMM due to IS is difficult to assess [4]. Previous reports including PMM due to hemorrhagic stroke showed that PMM of the leg was rarer than that of the arm (14% vs. 86% [11], 28% vs. 72% [1], 33% vs. 67% [2]). However, one DWI study showed that the frequencies of leg and arm PMM were 54% and 46%, respectively, suggesting that leg PMM might not always be less common than arm PMM [4]. Most studies did not examine the differences in frequencies of arm and leg PMM as they primarily focused only on PMM cases involving the arm, focusing on precentral knob infarctions, and did not include leg PMM patients. Hemorrhagic stroke can also cause PMM [1, 2, 11, 13, 18-20], albeit at a lower rate than IS. As an example, Figure 1A shows traumatic intracranial hemorrhage presenting as leg PMM.

LESION TOPOGRAPHY AND CLINICAL FEATURES

IS of the cortical surface is more likely to cause PMM than deeper brain lesions, which frequently cause pure motor hemiparesis; this is due to the compaction of descending motor axons in a small area in the subcortex, internal capsule, and brainstem [4]. In this regard, cortical infarction of the hand motor area is the most frequent site affected by arm PMM, whereas cortical infarction of the anterior cerebral artery (ACA) territory is the most frequently reported site involved in leg PMM [1, 2].

Table 1. Reported frequency of pure motor monoparesis due to ischemic stroke

References	Year	Modality	Frequency and lesion locations
Nelson et al. [107]	1980	CT	1 (1 arm) of 26 pure motor stroke. Lesion: no visual lesion.
Donnan et al. [86]	1982	CT	1 (1 leg) of 69 pure motor stroke. Lesion: posterior limb of internal capsule.
Rascol et al. [85]	1982	CT	1 (1 leg) of 30 pure motor stroke. Lesion: internal capsule infarction widespread to putamen and caudate nucleus.
Weisberg [8]	1984	CT	4 (leg or arm is not described) of 40 patient with small superficial cerebrovascular lesion.
Arboix et al. [87]	1990	CT/MRI	8 (4 arm and 4 leg) of 227 patients with lacunar infarctions (8 of 125 pure motor stroke). Lesion: 1 of 8 had internal capsule (arm or leg is not described) 7 had no visual lesion.
Borten and Lodder [10]	1991	CT	7 (5 arm and 2 leg) of 252 patients with first-ever supratentorial ischemic stroke (2 had slight sensory disturbance). Lesion: 6 of 7 had cortical infarctions. 1 patient had no visual lesion.
Chimowitz et al. [105]	1991	CT/MRI	11 (leg or arm is not described) pure motor monoparesis of 81 patients with pure motor, sensorimotor, or pure sensory stroke (11 of 48 pure motor stroke). Lesion: this report showed no details of lesion topography.
Chamorro et al. [78]	1991	CT/MRI	2 of (2 arm) 337 patients with lacunar stroke (sensory disturbance of these 2 patients were not described).
Melo et al. [11] *	1992	CT/MRI	29 (25 arm and 4 leg) of 255 acute stroke patients.

				Arm lesion: deep infarcts 2, superficial infarcts 12, hemorrhagic stroke 1, and no visible lesion 10. Leg lesion: deep infarcts 1, superficial infarcts of ACA 2, no visible lesion 1.
Mohr et al. [12]	1993	CT	8 (all arm) cases of 183 with cerebral convexity infarction of the MCA territory. Lesion: mid-Rolandic region.	
Castaldo et al. [15]**	2003	CT/MRI	35 arm of 4818 ischemic stroke patients. Lesion: all had MCA territory infarction including parietal lobe.	
Maeder-Ingvar et al. [1]*	2005	CT/MRI	153 (123 arm, 30 leg) of 4802 stroke patients. Arm lesion: MCA 59%, subcortex 26%, brainstem 9%, ACA 2%, others 4%. Leg lesions: ACA 43%, subcortex 27%, MCA 23%, brainstem 7%.	
Paciaroni et al. [2]*	2005	CT/MRI	51 (34 arm, 17 leg) of 2003 stroke patients. Arm lesion: subcortical (corona radiata, centrum semiovale) 35.3%, cortical branches of anterior superficial MCA 26.5%, deep perforators of MCA 23.5%, thalamocapsular 8.8%, and cortical branches of posterior superficial MCA 5.9%. Leg lesion: cortical branches of the ACA 23.5%, watershed infarcts 17.6%, thalamocapsular 17.6%, striatocapsular 11.8%, pons 11.8%, subcortical (corona radiata, central semiovale) 5.9%, deep perforators of MCA 5.9%, and cortical branches of the anterior superficial MCA 5.9%.	
Celebisoy et al. [44]**	2007	MRI	7 hand of 815 patients who were hospitalized in the stroke care unit (this report did not described sensory symptoms). Lesion: posterior portion of the precentral gyrus.	
Alstadhaug and Sjulstad [40]***	2013	MRI/CT	13 hand of 866 ischemic stroke patients (15 had hand paresis and remaining 2 of 15 had sensory disturbance, not PMM). Lesion: this study was limited to hand motor area.	

CT, computed tomography; MRI, magnetic resonance imaging; ACA, anterior cerebral artery; MCA, middle cerebral artery.

*These studies included hemorrhagic stroke.

**These studies focused only on upper limb monoparesis, and the research criteria did not include leg monoparesis.

***This study focused only on hand paresis due to hand knob area infarction, and other topographies or leg monoparesis were not included. In this study, only one patient underwent CT, and the remaining patients underwent diffusion weighted imaging.

Note: 34 of 35 patients underwent MRI in the study by Castaldo et al. [15].

Table adapted from Hiraga [4].

Additionally, isolated distal, proximal, and distal-predominant monoparesis cases are observed more frequently in cortical PMM than in subcortical PMM. Selective damage to primary motor cortex results in restricted paresis of the contralateral upper and lower limbs in patients with cortical PMM. Pyramidal signs vary in each case but are relatively infrequently seen in both cortical and subcortical cases of PMM due to IS [4].

This table does not contain the study by Timisit et al. [21], which reported five patients with hand palsy among 690 patients hospitalized in the stroke care unit. All five had sensory disturbances. This table also does not include the study by Peters et al. [35] that was limited to hand knob area infarctions presenting with hand paresis and used diffusion-weighted imaging for all patients; they found that 29 of 3499 patients treated in the stroke care unit had hand paresis and that 22 of 29 patients showed isolated hand paresis. However, 10 of these 29 patients showed sensory disturbances; thus, the exact frequency of PMM was not determined.

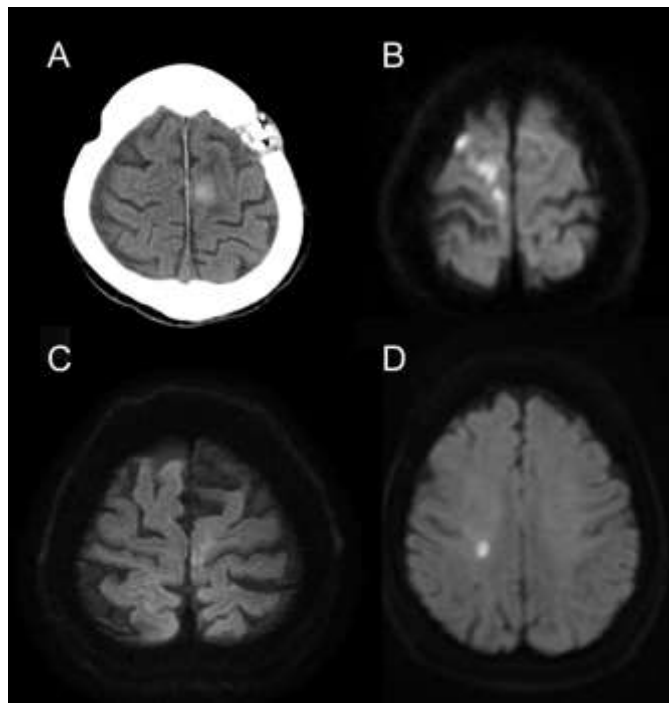


Figure 1. Neuroimaging studies of patients with pure motor monoparesis (PMM) of lower extremities. (A) Computed tomography of a patient with PMM of the right leg due to traumatic intracerebral hemorrhage located in the left leg motor area. (B) Diffusion-weighted images (DWI) of a patient with PMM of the left leg due to acute cerebral infarction located in the right anterior cerebral artery (ACA) territory. (C) DWI of a patient with PMM of the right leg showing acute infarction located in the left ACA territory. This case presented with monoparesis restricted to the foot. (D) DWI of a patient with left leg PMM due to acute cerebral infarction located in the right central semiovale (D).

Cerebral Cortex

Arm

Superficial [8, 10, 13] and cerebral convexity infarctions (mid-Rolandic lesions) [12], infarctions of the anterior and posterior superficial middle cerebral artery (MCA) territories

[2, 11], and infarctions of the pial branches of MCA [9] have been reported as causes of upper limb PMM.

The areas affected in PMM or upper limb monoparesis due to cortical infarction are the precentral gyrus, especially the precentral knob, and the parietal lobe; the former is more frequently reported. One study reported six patients with isolated hand palsy due to IS of white matter of the angular gyrus [21]. All patients had sensory symptoms/disturbances, and one had aphasia; none fulfilled the criteria for PMM, suggesting that the parietal lobe was involved in motor control of the hand. Another study of 35 upper limb PMM cases reported that all patients had infarctions in the MCA territory [15]; nine of the 35 cases had some involvement of both the precentral and postcentral gyri, and 17 had some postcentral gyrus involvement. Importantly, all patients exhibited some involvement of the parietal lobe infarct, indicating that the parietal lobe was important for PMM development. The inferior parietal lobe may also be important in PMM [22].

Numerous studies showed precentral knob infarctions presenting as PMM or upper limb monoparesis [22-42]. Some reports found that the causative lesions were in the posterior portion of the precentral gyrus (i.e., the anterior bank of the central sulcus) [30, 32, 43, 44], whereas others indicated the frontal lobe, precentral gyrus [45, 46], or motor cortex [47]. Most studies showed that patients with upper limb PMM due to precentral gyrus infarctions had either distal or distal-dominant weakness, whereas several cases presented with proportional weakness [13]. In a study of 12 patients with predominant weakness in specific fingers and small cortical infarct, two of whom had PMM [29], MRI revealed that the lesions were related to predominant involvement of ulnar fingers and were located significantly more medially than those associated with predominant involvement of radial fingers in the presumed hand representation area of the motor cortex. These observations support traditional views [48], although individual variations in motor topography were also found [29, 31]. Others showed that ulnar or radial fingers were predominantly affected in monoparesis due to cortical infarctions [22, 23, 28, 30, 32, 43, 44, 46]. Furthermore, some studies indicated ulnar–medial and radial–lateral correlations [28, 32, 43]; patients with large lesions had uniform finger involvement [28]. Likewise, PMM due to cortical infarctions, mimicking radial or ulnar nerve palsy, a condition resulting from precentral knob infarction that imitates anterior interosseous nerve palsy, was also reported [37].

In 1999, Schieber and colleagues emphasized that they identified no cases with individual digit paresis due to IS in which the greatest weakness was in the index, middle, or ring fingers [43], providing little evidence that individual digits were represented in separate cortical territories; instead, these findings suggested broadly overlapping (i.e., lateromedial) gradients in the cortical representation of radial and ulnar fingers. Although cases with isolated thumb [34] or index finger paralysis [31, 41] and those with isolated ring finger palsy [38] due to precentral knob infarctions were reported, there are no reported cases with isolated middle finger or little finger PMM due to IS. However, predominant thumb weakness was noted in some cases; one patient with significant thumb weakness during the recovery phase of hemiparesis exhibited predominant thumb flexion weakness [49], whereas another patient with isolated fingers weakness due to precentral gyrus infarction had predominant thumb weakness wherein thumb adduction and flexion were strongly affected [23]. These two cases, in addition to an isolated complete thumb paralysis case [34], provide supporting evidence that motor hand areas might contain a subregion that selectively represents the thumb [43]. Moreover, two previously reported cases with isolated index finger weakness [31, 41] and

one patient with prominent index finger weakness sparing the thumb and largely sparing the ulnar fingers [33] suggest the presence of a subregion selectively representing the index finger.

Although most cases of upper limb PMM due to cortical infarctions present with distal-dominant weakness, cases of isolated shoulder paresis were also reported [4, 50-54]. The first reported isolated shoulder PMM case in 2003 had an infarcted region restricted to the base of the central sulcus and in the deep white matter, located midway between the precentral knob and longitudinal cerebral fissure [50]. One patient with an isolated shoulder seizure due to a small cortical infarction had a lesion in a similar region [55], indicating that this location correlated with human shoulder motor somatotopy. Cases with PMM limited only to shoulder and arm muscles also had precentral gyrus infarctions in the precentral gyrus, medially to the precentral knob [56]. Although patients presenting with an affected wrist flexor were also diagnosed with PMM [25], predominant wrist extensor involvement was more frequently observed [26, 27, 44]. Furthermore, not many differences between finger extensors and flexors occur with precentral knob infarctions, except for mild differences observed in some cases [30, 44]. Deep tendon reflexes (DTRs) of the affected limb in PMM cases with precentral knob infarction were either normal or symmetric in some patients [22, 27, 37]. In particular, normal or symmetric DTRs were evident in patients with weakness in isolated or specific fingers [25, 31, 33, 34], as well as in those with isolated shoulder weakness [50, 51, 53]. However, several patients exhibiting weakness in specific fingers [29] and one patient with isolated shoulder palsy [54] had active DTRs of the affected limb. Other case reports also showed active or brisk DTRs [24, 28, 42, 43]. The Babinski sign was negative in most cases; thus far, only one case presenting with a positive Babinski sign has been reported [22]. In one study on cortical infarction presenting as arm PMM, patients demonstrated all DTR patterns: symmetrical, active, or decreased [13]. A case of claw hand deformity in acute stage of PMM due to hand knob area infarction mimicking peripheral nerve disease has been reported [42], although the majority of stroke-associated PMM cases did not exhibit any hand deformities in the acute stage. The differences in PMM and monoparesis with sensory disturbances might partially be due to postcentral gyrus involvement, as those with infarct regions involving both the precentral and postcentral gyri had sensory symptoms [25, 32]. Figures 2A and 2B show a patient with precentral knob infarction presenting as isolated hand PMM. Figure 2C shows a patient with radial-dominant hand PMM due to a precentral knob infarction, and Figure 2D shows a patient with proximal-dominant upper limb PMM due to cortical infarction.

Leg

Cortical branches of the ACA are the most common sites affected in leg PMM [2]. Classically, leg-predominant weakness due to stroke results from ACA territory infarctions and consequent paracentral lobule involvement [57]. Leg PMM due to cortical infarctions occurs in the precentral gyrus [58-67], paracentral lobe [68-73], and supplementary motor area [74]. Other small cortical infarctions in the ACA territory were also reported [10, 11]. Although the details of infarcts were not described, four patients with leg PMM due to occlusion or severe stenosis of internal carotid artery were reported [75].

The weakness patterns of leg PMM due to ACA territory infarctions can be proportional [68, 69], distal-dominant [59-62, 64-67, 72, 74], or proximal-dominant [59, 63, 73]. The rear portion of the medial precentral gyrus is the site affected in distal-dominant weakness [59].

One patient was reported to develop isolated toe paralysis in the recovery phase of a paracentral lobule infarction [72]. Additionally, several cases presenting with isolated foot drop due to precentral gyrus infarctions were reported [62, 64-67, 74]. These reports indicate that a small ACA territory infarction may cause isolated distal leg PMM. Some patients were reported to exhibit active DTRs of the affected leg [60,64,69], whereas normal or symmetric DTRs were observed in other cases [61-68, 72-74]; one patient with a decreased DTR was also reported [61]. A positive Babinski sign on the affected side was demonstrated in several cases [64, 69, 72, 73], whereas it was negative in others [60-65, 67, 74]. Figures 1B and 1C show ACA territory cortical infarctions presenting as leg PMM; the case illustrated in Figure 1C presented with isolated foot paresis.

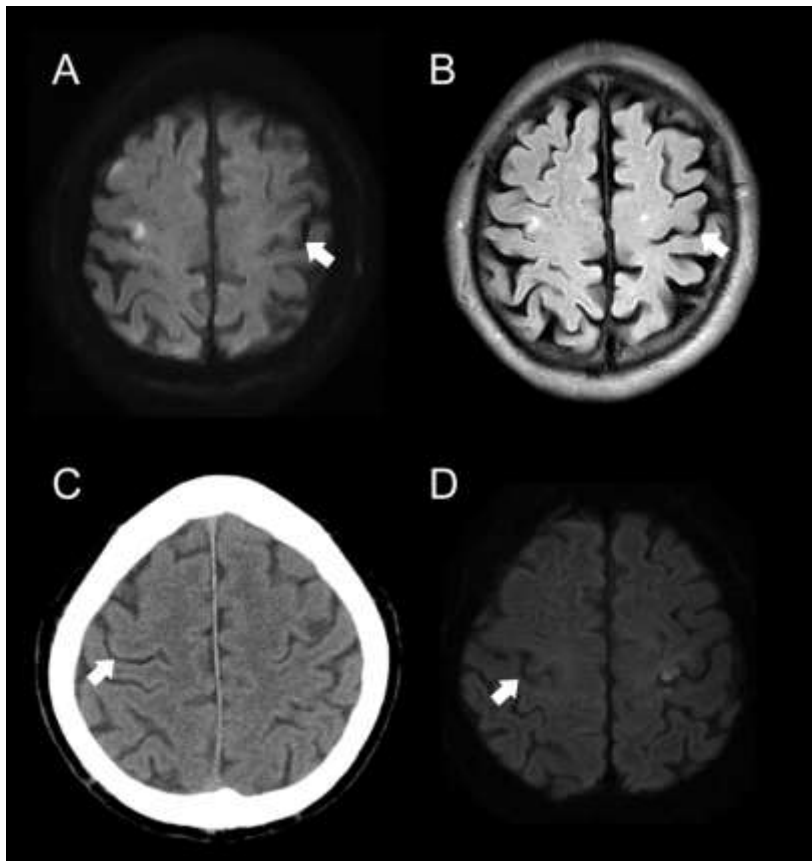


Figure 2. Neuroimaging studies of patients with pure motor monoparesis (PMM) of upper extremities. (A) Diffusion-weighted images (DWI) of a patient with PMM of the left hand due to an acute cerebral infarction located in the hand motor area. (B) Fluid-attenuated inversion recovery images showing an infarction of the same area. (C) Computed tomography of a patient with PMM of the right hand. This case presented with predominant involvement of radial fingers, and the affected region was located in the lateral region of the presumed hand representation area of the left motor cortex (precentral knob). This case indicated ulnar-medial and radial-lateral correlations. (D) DWI of a patient with proximal dominant-PMM of the right upper extremity, showing an infarction located in the left precentral gyrus medial to the knob-like structure, and laterally to the shoulder motor area. Arrows indicate the contralateral precentral knob.

Subcortex, Centrum Semiovale, and Corona Radiata

Although some patients with cortical infarctions presenting as PMM also had adjacent subcortical regions that were infarcted, a subcortical infarction without affected cortical regions can also lead to arm or leg PMM. A large study showed that 26 patients with arm PMM and 27 patients with leg PMM had subcortical infarctions [1]. Another large study showed that ten patients with subcortical (corona radiata or centrum semiovale) infarctions had PMM; most cases had PMM of the arm [2]. A report on subcortical infarctions of the superficial MCA territory affecting the centrum semiovale showed that 2 of the 36 patients had upper limb monoparesis [76]. Other reports presented cases with frontal subcortical infarctions in the ACA territory that presented as leg PMM [68, 77]. A case report showed that brachial monoparesis in a patient was due to a corona radiata infarction [78]. Another study including eight arm PMM and six leg PMM patients [79] noted that patients with leg PMM had more posteriorly and medially located infarct regions than those with bulbofacial paresis or arm PMM, indicating anterolateral to posteromedial distribution. Other studies also indicated anteroposterior somatotopy in the corona radiata; leg fibers were located more posteriorly than arm fibers [68, 80, 81]. One diffusion tensor tractography (DTT) study showed that the hand fiber tract was located in the anteromedial portion of corona radiata [82], while another DTT report demonstrated that hand somatotopy of the corticospinal tract (CST) was located anterolaterally to the leg somatotopy of the CST in central semiovale [83]. These DTT findings and PMM case studies indicate that hand fibers are located more anteriorly than leg fibers and that anterior corona radiata infarction might manifest as hand PMM. PMM involving one or more fingers due to subcortical infarction was rarely reported. However, a patient with palsy of only ring and small fingers due to a subcortical infarction was reported [84]. Moreover, another patient had profound weakness and decreased sensation in the thumb and index finger due to a corona radiata infarction [46]; this patient was not diagnosed with PMM due to accompanying sensory disturbances. However, these observations indicate that involvement of specific fingers can occur not only due to a cortical infarction, but also due to a subcortical or corona radiata infarction, suggesting finger somatotopy might also exist in the subcortical region.

Patients with ACA territory subcortical infarctions and distal-dominant [77] or proportional [68] weakness were reported to have normal DTRs. Additionally, cases with leg PMM due to corona radiata infarctions had proportional weakness with brisk or normal DTRs of the affected leg [68]. Figure 1D shows a subcortical infarction presenting as leg PMM.

Internal Capsule

A CT study showed that leg PMM could result from an internal capsule infarction spreading to the putamen and caudate nucleus [85] or from an infarction located just lateral to the posterior limb of the internal capsule [86]. In another report, PMM due to an internal capsule infarction was detected by MRI [87], although it was not clear whether the arm or the leg was affected. Yet another report suggested that isolated arm monoparesis might also be due to infarction of the internal capsule [78]; however, no information was enclosed regarding sensory disturbances in that study. Other cases involving infarction of the posterior limb of the internal capsule presenting as leg PMM were also reported [59, 68]. Moreover, one report described a patient with infarction of the posterior limb of the internal capsule who developed an abrupt fall but showed only unilateral pyramidal tract signs in lower extremities during neurological examination [88].

A previous large study on monoparesis found that internal capsule infarctions, either striatocapsular or thalamocapsular, led to leg PMM in a small number of patients [2]. Traditionally, the somatotopic CST organization in the internal capsule is a homunculus, i.e., the hand fibers are located more anteriorly and slightly medially to the foot fibers. However, considerable overlap was demonstrated between hand and foot fibers [89]. A linear infarction along the long axis of the internal capsule was characteristic of posterior limb internal capsule infarction presenting as leg PMM [68]. These two cases support the results of an MR tractography study [90, 91], which demonstrated that CST foot fibers in the posterior limb of internal capsule might be somatotopically posteromedial to the hand fibers along the short axis of internal capsule. However, other studies showed that hand fibers were located anteromedial to foot fibers in the posterior limb of internal capsule [82, 92].

Clinically, two reported cases with leg PMM due to internal capsule lesions had slight proximal-dominant weakness with no Babinski sign and normal DTRs [68], whereas a separate patient had proportional leg weakness [59].

Brainstem

An acute brainstem infarction is difficult to detect by CT; thus, few brainstem infarctions presenting as PMM by CT were reported. One earlier CT study presented a patient with arm PMM due to a pontine infarction [93], whereas a larger study showed only two leg PMM cases resulting from pontine infarctions, with no patients presenting exhibiting arm PMM [2]. Another study showed nine arm PMM and seven leg PMM cases due to brainstem infarctions [1]. Distal-dominant brachial monoparesis results from infarction of the ventromedial pontine base on DWI, indicating that arm fibers are located more medially than leg fibers [94]. Rostral lateral pontine infarction presenting with leg PMM in three patients [95] indicated that the lesions responsible for crural monoparesis were presumably located in the dorsal and lateral regions of pontine base. Another study indicated that the lesions in PMM patients with leg-predominant weakness were dorsal and lateral in the pons [59]. The regions for pontine infarctions that did not present with PMM were located in the ventromedial region in patients with lower predominant hemiparesis, and were correlated with lesions in the dorsomedial and dorsolateral portions in patients with upper predominant hemiparesis [80], whereas the upper extremity fibers were located more ventromedially than the lower extremity fibers in four patients with brachial monoparesis and dysarthria due to pontine base infarctions [96]. A voxel-based MRI analysis of the basilar pons [97] indicated that upper extremity weakness was correlated with specific pontine regions; however, another study [98] did not show significant topographical differences between patients with paresis predominantly affecting the arm or leg, suggesting that the topographical arm/leg distribution was disrupted in the pons and that PMM due to brainstem infarctions was rare. However, DTT studies indicated the presence of motor somatotopy of the hand and leg in the brainstem. Specifically, hand somatotopy of the CST is located in the anteromedial portion of pons and leg somatotopy of the CST is located posterolateral to hand somatotopy of the CST [82, 99]. Additionally, hand somatotopy of the CST is located in the medial portion of the medullary pyramid, whereas leg somatotopy of the CST is situated in the lateral portion of the medullary pyramid [100]. The case report also suggests that the CST fibers innervating lower and upper extremities decussate at different levels in the lower medulla oblongata [101].

PMM due to midbrain or medulla infarctions is extremely rare. There are several case reports of patients with cerebral peduncular or medial medullary infarction presented with

monoparesis; however, these cases either had accompanying sensory symptoms or did not have detailed description of symptoms [59, 102, 103]. One Japanese study reported a patient with PMM due to a medullary infarction that was limited to the upper lateral region of medullary pyramid [104]. PMM due to lateral medullary infarction was also reported by a group from Japan [101].

Assessment of clinical features reveals that patients with arm PMM due to pontine infarctions develop proportional weakness with normal DTRs [93] or distal-dominant arm weakness with active DTRs [94], both in the absence of a Babinski sign. Among patients with leg PMM due to rostral lateral pontine infarctions, two cases with dorsolateral infarcts were proximally dominant, and one case with an anterolateral infarct had proportional weakness [95]. One patient with a medullary infarction presenting as leg PMM showed proportional weakness with active DTRs [104], and two patients with leg PMM due to medullary infarctions had a positive Babinski sign in the affected leg [101, 104].

MECHANISMS OF STROKE IN PMM

PMM frequently occurs following cortical infarctions, and findings from earlier reports suggest that PMM rarely results from lacunar infarctions [11, 13, 105]. Eleven patients with leg or arm monoparesis revealed that the etiology of stroke was occlusive large artery disease and cardioembolism in six and three cases, respectively; the cause was either undetermined or miscellaneous in the remaining two patients [105]. Melo et al. reported 28 patients with PMM due to IS; the causes were determined as deep infarcts in 3 patients, superficial infarcts in 14 patients, and no visible lesions in 11 patients [11]. In contrast, a large study indicated that the etiologies of PMM due to IS were small artery disease, cardioembolism, and atherosclerosis in 39.2%, 15.7%, and 9.8% of patients, respectively [2], suggesting that small artery disease was the most likely cause of PMM. Another large study found no particular etiology in arm or leg monoparesis subgroups [1].

A study of seven patients with superficial infarctions and monoparesis (leg, 2; arm, 5) revealed cardioembolism as the cause in two cases; however, neither had significant carotid stenosis [10]. Another report showed that, out of 14 patients, arm PMM or monoparesis due to IS resulted from carotid pathology and cardioembolism in six and four cases, respectively [13]. In studies that found a correlation between upper limb monoparesis and parietal lobe infarction, all six patients had infarctions in the vascular border zone of angular gyrus white matter; five of these six cases had carotid stenosis, suggesting a hemodynamic mechanism [21]. Others showed that cardiac sources were the most frequent etiologies: cardioembolism/cardiac source in 46%, large artery atherosclerosis/carotid stenosis in 34%, MCA branch thrombosis in 17%, and cryptogenic in 3% [15]. In a study of 11 patients with distal arm PMM due to cortical infarctions, three had a patent foramen ovale, three had carotid stenosis, and two had cardiac arrhythmias; one of the latter also had MCA stenosis [28]. A study of six patients with isolated upper limb weakness due to cortical infarction reported cardioembolism, large-artery atherosclerosis of the MCA, and undetermined etiology in one, two, and three cases, respectively [22]. In a report of 29 precentral knob infarctions (22 of 29 patients showed isolated hand paresis, and 10 of 29 patients showed sensory disturbance), ten patients had carotid stenosis, whereas four were found to have a cardiac

embolic source [35]. These results indicated that precentral knob area infarctions were frequently associated with atherosclerotic changes in the carotid artery and suggested an arterioarterial thromboembolic stroke mechanism. Yet in another study of seven patients with arm PMM due to precentral gyrus infarctions, the mechanism of stroke was determined as large artery atherothrombosis (internal carotid artery stenosis, 2 cases), cardiogenic embolism (2 cases), secondary to hypoperfusion (1 case), and undetermined in (2 cases) [39]. A recent large study including patients with hand PMM due to knob infarctions had 15 patients with hand palsy, two of whom had sensory disturbances. In that study, stroke etiologies were large artery atherosclerosis in three patients, cardioembolism in two patients, small artery disease in five patients, and unknown in five patients [40]. Kim proposed that the motor cortex region representing ulnar fingers might be a border zone between large arteries. The author suggested that predominant involvement of ulnar fingers due to cortical infarctions was closely associated with severe proximal arterial stenosis or occlusion of the MCA or internal carotid artery and that predominant involvement of radial-sided fingers was often related to emboligenic stroke [29]. Other case reports showed carotid stenosis [34, 36, 45] or cardiogenic embolism [31, 37] as underlying stroke etiologies in PMM patients. One case report showed that hypotension might either cause or contribute to the precentral knob infarction in a patient with hand PMM [26]. The first reported isolated shoulder palsy due to a small cortical infarction with severe carotid stenosis was reported as a border-zone infarction [50]. In fact, proximal arm weakness has traditionally been considered a manifestation of border-zone infarction. However, subsequent isolated shoulder palsy cases due to cardiogenic embolism [51, 54] and atherothrombosis [52, 53] indicated that the mechanism of stroke in arm PMM due to cortical infarction might include both cardioembolic and carotid sources, which underlies the difficulty in determining the more frequent etiologies in these clinical entities. Rare etiologies reported by several groups include aortogenic embolism [41] and cerebral angitis [42] presenting as precentral knob infarctions and PMM.

One study including six patients showed that the mechanism of leg PMM due to small ACA cortical infarctions was cardiac embolism in three cases; the etiology was unknown in the remaining three patients [64]. Other reports also showed carotid stenosis or carotid emboli [8, 59, 63, 73], ACA stenosis [67], and cardiogenic embolism [59, 68] as causes of leg PMM due to ACA cortical infarctions. One study showed that the majority of leg PMM cases were correlated with occlusion or severe stenosis of the internal carotid artery [75]. Additionally, a large study showed that watershed infarctions were found only in patients with leg PMM but not in those with arm PMM [2].

Centrum semiovale infarcts with upper limb monoparesis were reported to result from small vessel disease [76]. Furthermore, lacunar infarctions of the internal capsule were reported to cause PMM [59, 86, 87]. Small artery disease might be the most frequent mechanism for brainstem infarctions [94, 95]; however, it can also result from vertebral artery occlusion [101].

DIFFERENTIAL DIAGNOSIS

As described above, either the radial or ulnar side can be predominantly affected in cortical hand PMM, raising the possibility of misdiagnosis of these patients with cervical disc

disease or radial or ulnar neuropathy [5]. It is critical to include cerebral lesions in the differential diagnosis of monoparesis. Following are the important points for a successful diagnosis of PMM due to IS [4]: (1) *Anamnesis*. Typically, mononeuropathy, such as radial nerve palsy, has a preceding compression event; thus, sudden or acute onset of weakness in one hand without history of a compression event might be an indicator of PMM due to IS. (2) *Risk factors for cerebrovascular disease*. The presence of hypertension, diabetes mellitus, atrial fibrillation, and/or hyperlipidemia is also important. (3) *Pyramidal signs*. DTRs are often decreased and no pathological reflexes are present with peripheral nervous system damage. Therefore, DTRs and muscle tone are important tools to distinguish central and peripheral weakness. However, PMM due to IS does not always show active DTRs in the affected limbs. In fact, there is a relatively low frequency of active DTRs in both arm and leg PMM cases resulting from IS [13, 68], and the absence of pyramidal signs should not exclude PMM due to IS. (4) *The distribution of weakness*. Patients with PMM due to IS show frequent distal-dominant weakness, and some have radial or ulnar finger-predominant weakness. In these patients, the affected muscles are not completely the same as the peripheral nerve distribution, and careful neurological evaluation is necessary. (5) *Neuroimaging*. DWI is the most useful tool for diagnosis, as CT and conventional MRI cannot distinguish between acute and chronic lesions. In fact, 11 of 28 patients with PMM due to IS had no visible lesions by CT or conventional MRI [11], and all eight patients with PMM in one study showed no visible lesions on repeat CT scans [87]. In a study of distal arm PMM patients, 77% of the 35 patients demonstrated no causative lesions on their first CT scans [15]. Without the use of DWI, small cortical infarctions as well as acute infarctions might be overlooked or misidentified. The recommended MRI sequences include DWI for acute ischemia, fluid-attenuated inversion recovery, T2*-weighted gradient-echo images for bleeding, and MR angiography.

PROGNOSIS

A large study in 2005 showed that 41% of PMM patients returned to their former activities and that 41% of patients required some assistance, indicating that outcomes of PMM are generally favorable [1]; however, isolated facial paresis and hemorrhagic stroke were observed in some patients. Therefore, the exact outcomes of PMM due to IS remain unclear.

Some case reports on cortical infarctions presenting as arm PMM described improvements [14, 24, 42, 56]. While most studies did not evaluate the prognosis of PMM due to IS [8, 11, 12, 25, 27, 32, 36, 38, 43, 44, 50], others determined good or complete recovery in patients with PMM due to IS [10, 15, 22, 23, 26, 28-31, 33-35, 37, 41, 45, 46, 51-53, 106]. For example, one study determined that 14 of the 35 patients with arm PMM due to cortical infarctions had complete clinical recovery, albeit residual mild weakness was reported in 21 patients [15]. Yet another report showed that all patients with PMM had complete recovery within 2–4 weeks [10]. Furthermore, several case studies showed a high frequency of complete recovery [22, 28, 35]. Finally, patients exhibited good recovery despite the presence of severe finger weakness at initial examination [23, 29, 34, 37, 45].

The rate of recurrent stroke in PMM due to IS has not been established; however, several studies examined outcomes in patients with PMM of the arm. Specifically, one report on arm PMM due to cortical infarction showed a 14% rate of recurrent stroke (mean follow-up, 1.7 years) [15], whereas another study of seven patients with arm PMM due to cortical infarctions with a mean follow-up of 14 months showed two patients had stroke recurrence. The second study also reported the death of one patient; the remaining four cases had excellent recovery with no recurrence [39]. A recent study of 15 patients with knob infarctions, of which 13 had PMM and 2 had monoparesis with sensory disturbances, had a mean follow-up period of 29.8 months [40]. While none of the patients had recurrence, one patient died, and two patients had bad recovery; the hand function was good and fair in six and eight cases, respectively [40]. In some studies, patients with PMM underwent carotid endarterectomy for carotid stenosis [15, 34, 36, 45]. Finally, patients with isolated shoulder PMM due to cortical infarction were reported to have good recovery [51-53]; however, in one study, one patient died on day ten [54].

In a study of cortical ACA infarction cases presenting with lower limb PMM, two of the three patients with proximal weakness had good clinical recovery, whereas the only patient with distal weakness had poor recovery [59]. A separate report showed that five of the six patients with distal leg PMM had partial or complete clinical recovery [64]. Other studies reported recoveries ranging from complete [62, 66] to good [70, 72] or improved [60, 61, 63, 65, 67, 69, 74]. One patient with a subcortical infarction had a good prognosis [77]. Prognoses have not been described for patients with subcortical, centrum semiovale, or corona radiata infarctions presenting as PMM. Patients with pontine infarctions presenting as PMM can have good recoveries; three cases with leg PMM due to rostral lateral pontine infarctions showed good functional recovery [95], and one case with brachial PMM due to a pontine infarction showed a complete recovery [93]. Furthermore, gradual improvements or complete recovery were observed in other cases with brachial PMM due to pontine infarctions or with leg PMM due to medullary infarctions [94, 101, 104].

In summary, previous studies have indicated a good prognosis for patients with arm PMM due to cortical infarctions, especially those in the precentral knob. Due to their rarity, the outcomes of patients with PMM due to infarctions in other regions are difficult to discuss; however, most reported cases had good clinical recoveries. Ultimately, both short- and long-term outcomes depend on risk factors and stroke mechanisms [40].

FURTHER INVESTIGATION

The history of studies about PMM due to IS provides anatomical evidence regarding motor cortical representations in human cortex and differences between motor fiber pathways of the arm and leg; however, some anatomical questions remain debatable. Tractography studies were not always consistent with clinical experiences in patients with PMM due to IS. Although some individual variation exists, it is unclear whether motor somatotopy of the arm and leg clearly exists or breaks down in the deeper regions, especially the brainstem. Moreover, whether individual fingers are represented in a separate cortical territory with overlap should be reexamined as certain case reports indicated that isolated thumb, index

finger, or ring finger palsies could be the sole manifestation of cortical infarction [4]. Finally, individual motor somatotopy of the toe was not well understood until now.

Despite its importance, PMM due to IS remains underestimated. In recent years, it has been increasingly reported by neurologists who recognize and understand this atypical stroke syndrome. However, orthopedists and general clinicians are mostly unaware of this condition, and neurologists should alert physicians to its presence. Two large studies from 2005 were hospital-based research and did not use MRI, especially DWI, in all patients. Moreover, the percentage of patients who had PMM in those studies was probably smaller than the general population as patients with very minor motor deficits are less likely to be admitted to a hospital [2] or are misdiagnosed with nonvascular causes of weakness. Additionally, the assumption that lacunar etiology is not likely a cause of PMM due to IS should be reexamined [2]. The exact frequency of PMM due to IS, its clinical features such as paresis pattern, DTR, and the presence of Babinski sign, and prognosis, especially the recurrent stroke rate, should be examined in a large study by a national survey, using DWI for all cases.

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Chapter 89

STROKE ASSOCIATED WITH NEPHROTIC SYNDROME - ACUTE ISCHEMIC STROKE, CEREBRAL VENOUS SINUS THROMBOSIS, AND INTRACEREBRAL HEMORRHAGE

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ABSTRACT

Nephrotic syndrome is defined by the presence of heavy proteinuria, hypoalbuminemia, hyperlipidemia and peripheral edema. Thrombotic diseases are also frequently observed. Arterial and venous thromboses are potential complications of nephrotic syndrome. Arterial thromboses are less frequent than venous thromboses and the most common locations are femoral arteries, although other arteries may be involved. Stroke associated with nephrotic syndrome has been reported in a number of case reports, but it is rare and its clinical details remain obscure. We identified ten cases of acute ischemic stroke, two cases of cerebral venous sinus thrombosis, and four cases of intracerebral hemorrhage by screening the hospitalized patients enrolled in our stroke unit. The incidence of acute ischemic stroke associated with nephrotic syndrome was 0.09% of all kinds of stroke and 0.12% of acute ischemic stroke, and was fivefold that of cerebral venous sinus thrombosis. The subtypes of stroke identified in our screening were large-artery atherosclerosis (six instances), small-vessel occlusion (three instances), and cardioembolism (one instance). The results of a retrospective cohort study showed that acute ischemic stroke-associated nephrotic syndrome is strongly associated with atherosclerosis of the cerebral artery, especially in the anterior circulation. Patients also exhibited atherosclerosis of the internal carotid and lower extremity arteries. The causal disorder of nephrotic syndrome was diabetic nephropathy in eight of these cases. Twenty-five patients with cerebral venous sinus thrombosis were admitted to our hospital during the same period. Nephrotic syndrome has been reported to be responsible for 0.6-1.0% of all cases of cerebral venous sinus thrombosis; however, our study showed that this percentage may be higher. Nephrotic syndrome essentially induced a hypercoagulable state. However, four cases of intracerebral hemorrhage associated with nephrotic

syndrome were identified by the screening. These exhibited the presence of strong risk factors for hemorrhage, including hypertension, vascular vulnerability with atherosclerosis or amyloidosis, and change of bloodstream after operation, suggesting that they overcame the risk for thrombophilia. The diseases associated with nephrotic syndrome were diabetic nephropathy and amyloidosis in three cases and one case, respectively. Nephrotic syndrome tends to be associated with a risk for arterial or venous thrombosis. In addition, we must pay attention to intracerebral hemorrhage associated with nephrotic syndrome in cases of stroke.

1. INTRODUCTION

Nephrotic syndrome (NS) is defined by the presence of heavy proteinuria (protein excretion greater than 3.5 g/24 hours), hypoalbuminemia (less than 3.0 g/dL), hyperlipidemia, and peripheral edema [1] (Figure 1). Thrombotic diseases are also frequently observed. Arterial and venous thromboses are potential complications of NS. Arterial thromboses are less frequent than venous thromboses, and the most common locations are femoral arteries, although other arteries may be involved [1-3]. Acute ischemic stroke (AIS) associated with NS has been reported in several case reports [4-10], but this is relatively rare.

Cerebral venous sinus thrombosis (CVT) is less common than most other types of stroke, but is recognized with increasing frequency due to the widespread use of magnetic resonance imaging (MRI) and rising clinical awareness. The clinical severity varies, and severe sequelae can occur when treatment is delayed. In some cases, death may occur from brain herniation due to increased intracranial pressure; therefore, quick and accurate diagnosis is required.

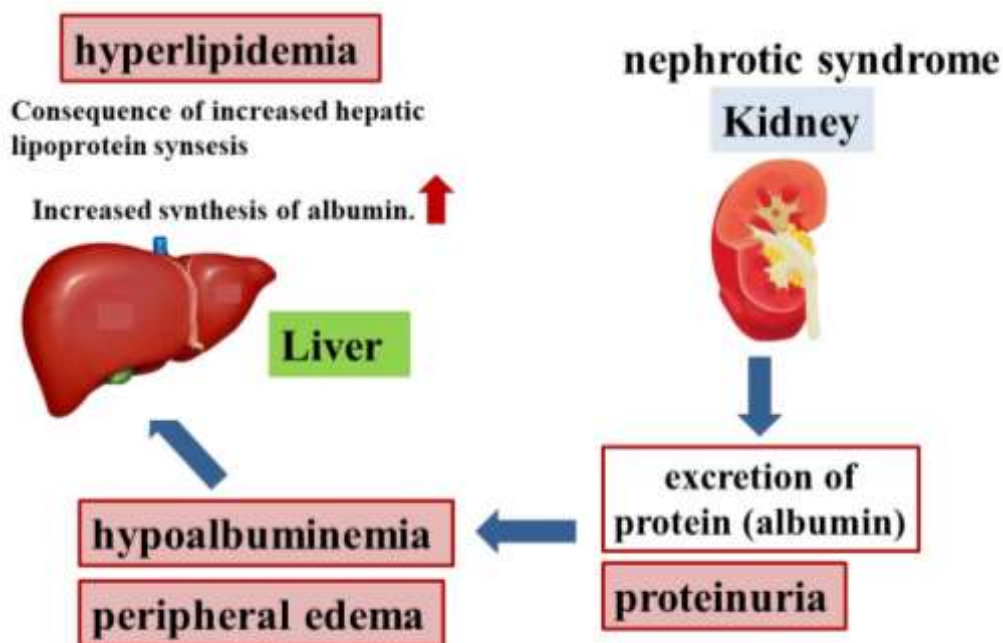


Figure 1. Clinical symptoms in nephrotic syndrome.

As CVT is also caused by various underlying diseases, it is important to analyze the causative disorders [2, 11, 12], as well as the immediate treatment for thrombosis. In the past, CVT was attributed to infections of the face and otomastoid areas, but since the introduction of antibiotics, it is more often related to pregnancy, puerperium, neoplasms, dehydration, genetic or acquired thrombophilia, and oral contraceptives [2, 11-13]. In Western countries in particular, CVT is increased by the hypercoagulability induced by oral contraceptive use [14-16]. NS is one of the risk factors for CVT. With recent increases in the number of patients with NS, it is likely that the number of patients with CVT caused by NS will increase, especially in Japan.

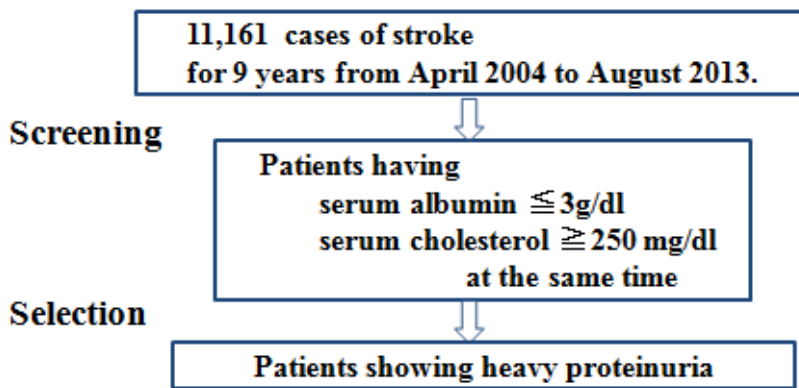
Hypercoagulability contributes to the predisposition to thromboembolism in NS. The low molecular weight coagulation factors (Factor IX, XI), antithrombin III, plasminogen, and free protein S excreted into the urine by the breakdown of the permselectivity barrier of the glomerular capillary wall have been reported, along with inverse increases in high molecular weight coagulation factors (Factor V, VII, VIII and X), fibrinogen, alpha 2-antiplasmin, alpha-2-macroglobulin and platelet aggregation [16-23]. These changes have been attributed to a hypercoagulable state in which an imbalance between procoagulant/prothrombotic factors and anticoagulant/antithrombotic factors promote thromboses in veins and arteries. Hypoalbuminemia, secondary volume depletion, and hyperlipidemia also could act as risk factors for thromboembolism or atherosclerosis. NS may independently predispose individuals to arterial and venous thromboembolism, but the exact mechanism of the hypercoagulability is as yet not fully understood [1-4, 16-23].

Hypercoagulability due to NS involves the pathogenesis of AIS or CVT. When we retrospectively screened stroke patients with NS from hospitalized patients enrolled in our stroke registry, it was found not only in those patients with AIS and CVT, but also, unexpectedly, in some patients with intracerebral hemorrhage (ICH) [24-26]. These patients had strong risk factors for ICH, suggesting that they had overcome the risk for thrombophilia. Attention should be paid to NS as a risk factor for stroke including AIS, CVT, and also ICH.

2. SCREENING FOR STROKE PATIENTS WITH NS

We retrospectively analyzed 11,161 cases of hospitalized patients enrolled in our stroke registry during a nine-year period from April 2004 to August 2013. The number of patients with serum albumin lower than 3.0 g/dl was 196 (1.9%) out of 10,057 patients examined, and that of patients with serum cholesterol higher than 250 mg/dl was 1,201 (11.3%) of 10,619 patients examined. The patients with albumin below 3.0 g/dl and serum cholesterol above 250 mg/dl at the same time were screened, and only 21 cases fulfilled both levels. From these patients, furthermore, the cases showing heavy proteinuria were selected. The proteinuria was estimated quantitatively by measuring the protein in a urine/24 hours or qualitatively by using a paper kit (Eiken Chemical Co. Japan) (Figure 2). They included two cases showing negative or equivocal ($\pm$) results in the protein paper kit test, one case with 1+ (30 mg/dl), two cases with 2+ (100 mg/dl), five cases with 3+ (300 mg/dl), and 11 cases with 4+ (1000 mg/dl). The 16 patients showed urinary protein 3+ or 4+ - i.e., heavy proteinuria - and comprised 10 patients with AIS, four with intracranial hemorrhage, and two with cerebral venous thrombosis (CVT) (Table 1). Nine of these 16 patients showed protein excretion greater than

3.5 g/24 hours. In the other seven patients, the content of protein in urine/24 hours was not measured. However, they all had moderate to severe peripheral edema and hyperlipidemia, and the diagnosis of NS was also strongly suggested in these seven cases.



Proteinuria: measured quantitatively by measuring the protein in a urine/24 hours or qualitatively by using paper kit (Eiken Chemical Co. Japan).

Figure 2. The screening and selection of the stroke patients with nephritic syndrome.

Table 1. The patients of acute stroke

	TIA	SVO	LAA	CE	other	ICH	SAH	Total
Patients n.	660	2196	2547	2023	235	2200	733	10619
① TC >250	60 (9.1)	299 (13.6)	373 (14.6)	133 (6.6)	17 (7.2)	242 (11.0)	75 (10.2)	1201 (11.3)
Patients n.	632	2070	2436	1958	202	2055	675	10057
② Alb < 3.0	3 (0.5)	16 (0.8)	44 (1.8)	66 (3.4)	9 (4.5)	48 (2.3)	8 (1.2)	196 (1.9)
Patients n.	263	886	1123	917	99	1226	477	5002
③ massive UP	8 (3.0)	21 (2.4)	40 (3.6)	45 (4.9)	6 (6.1)	118 (9.6)	33 (7.0)	272 (5.4)
① + ② + ③	0	3	6	1	2 (CVT)	4	0	

Abbreviations: TC – total cholesterol; Alb – albumin; UP – urinary protein; TIA – transient ischemic attack; SVO – small-vessel occlusion; LAA – large-artery atherosclerosis; CE – cardioembolism; ICH – intracerebral hemorrhage; SAH – subarachnoid hemorrhage; CVT – cerebral venous sinus thrombosis.

3. ACUTE ISCHEMIC STROKE (AIS) ASSOCIATED WITH NS

1) AIS Patients

The 10 cases – eight males and two females, 64.4 ± 8.6 yrs (Mean $\pm$ SD) – were diagnosed as AIS associated with NS. The incidence rate was 0.09% of all kinds of stroke (total 11,161 patients) and 0.12% of AIS (total 8,116 patients) in our hospital. Of these ten cases, six were of large-artery atherosclerosis (LAA), three of small-vessel occlusion (lacune, SVO), and one of cardioembolism (CE) by the TOAST criteria. On MRI and MRA, middle cerebral arteries, anterior choroidal artery, and internal carotid artery (occlusion) were

involved in six cases, one case and one case, respectively. SVOs were located in the supratentorial basal ganglia in all three of the SVO cases (Table 2). NS had not been diagnosed prior to admission in any of the 10 cases. Pathological diagnosis was arrived at through histopathological examination of the renal biopsied samples in two cases; amyloid nephropathy due to primary amyloidosis in one case, and membranoproliferative glomerulonephritis (MPGN) in one case. Eight cases were diagnosed as diabetic nephropathy (DMN) based on the long history of diabetic mellitus (DM) and the exclusion of other pathogenetic diseases, although renal biopsy was not performed (Table 2).

Table 2. The patients of acute ischemic stroke with nephrotic syndrome

Case	Age	Sex	Type	MRI Location of infarction	Alb	TC	UP	day	E	DM Duration (years)	NS type
1	50	F	LAA	R. MCA (BAD)	3	284	4	8.4	++	15	DN s/p
2	53	F	LAA	R. MCA	1.8	707	4	8.6	++	—	AMN
3	59	M	LAA	R. Ant. Choro. A	2.1	336	3	9.5	++	5	DN s/p
4	62	M	LAA	L. MCA	2.8	407	4	6.4	++	30	DN s/p
5	79	M	LAA	R. MCA (R. IC occlusion)	2.8	315	4		++	20	DN s/p
6	70	M	LAA	R. MCA,	1.9	326	3		+	10	DN s/p
7	65	M	SVO	L. Basal Ganglia	2.7	264	4	6.5	++	—	MPGN
8	68	M	SVO	R. Basal Ganglia	3	285	3		++	2	DN s/p
9	69	M	SVO	R. Basal Ganglia	3	299	3		++	4	DN s/p
10	69	M	CE	R. MCA	3	264	4		++	8	DN s/p

Abbreviations: TC – total cholesterol; Alb – albumin; UP – urinary protein, E – edema; SVO – small-vessel occlusion; LAA – large-artery atherosclerosis; CE – cardioembolism; L – left; R – right; MCA – middle cerebral artery; BAD – branched atheromatous disease; Ant – anterior; Choro. – Choroidal; IC – internal carotid; NS – nephrotic syndrome; DN – diabetic nephropathy; MPGN – membranoproliferative glomerulonephritis; AMN – amyloido nephropathy.

2) Arterial Stenosis in the Patients

Extent of atherosclerosis was evaluated by the atherosclerosis score of intracranial and extracranial arteries on MRA [27]. A total of 17 vessels – including the common carotid arteries, the extracranial and intracranial portions of the internal carotid arteries and the vertebral arteries, the anterior, middle, and posterior cerebral arteries, and the basilar artery – were evaluated by the cervical and intracranial MRAs. The individual total scores were divided into two scores: an anterior score and a posterior score, related to the anterior circulation vessels (common carotid, internal carotid, anterior cerebral, and middle cerebral arteries) and posterior circulation vessels (vertebral, basilar, and posterior cerebral arteries), respectively.

Ultrasonography of carotid arteries and lower extremities arteries was performed and the stenotic lesions of these arteries graded. The atherosclerosis of lower extremity arteries were also evaluated by measurement of ankle-brachial index (ABI). A total carotid ultrasonic score (T-CUS) and total extremities ultrasonic score (T-EUS) were estimated as the sum of the right

and left ultrasound score grades for the carotid and extremity arteries, respectively. A total ABI (T-ABI) score was estimated as the sum of the right and left ABI.

We carried out a retrospective cohort study to assess the association between NS and atherosclerosis progression. AIS patients with NS due to DMN were compared with AIS patients without NS. Cases were compared with controls in respect of body mass index (BMI), morbidity of hypertension, and several laboratory findings. Case 2 with amyloid nephropathy, case 7 with MPGN, and case 10 with CE, were excluded from this study. The remaining seven patients with AIS and NS due to DMN were selected as cases (NS group). Cases were compared with AIS patients with DM without NS (control group) who were matched to cases in a 2:1 ratio by age, sex, use of medications for DM, and HbA1c level. The control group included 11 cases with SVO and three cases with LAA, and had a larger number of SVOs than that of the NS group. The NS group had statistically significantly higher cerebral artery atherosclerosis scores, especially in the anterior circulation. The NS group also showed higher T-CUS, although there were no significant differences compared with the control group. T-EUS in the NS group were higher than those in the control group. However, there were no differences in T-ABI scores between the two groups. The NS group demonstrated hypoalbuminemia and hypercholesterolemia, as well as anemia, increased creatinine level and low eGFR, which were consistent with chronic kidney disease. They also had high fibrinogen and D-dimer levels, indicating a hypercoagulable state (Table 3, 4).

Table 3. Comparison of clinical and atherosclerotic characteristics between patients with acute ischemic stroke and nephrotic syndrome due to diabetic nephropathy (NS group) and patients with acute ischemic stroke and diabetes mellitus without nephrotic syndrome (control group)

	NS group	Control group	P
Patient (n)	7	14	
Age, y	65.3 ± 9.3	66.0 ± 8.0	—
Sex/male	6 (85.7)	12 (85.7)	—
DM	7 (100)	14 (100)	—
Duration	12.3 ± 10.1	7.9 ± 6.2	0.397
Hb A1c	8.7 ± 3.3	8.1 ± 1.7	—
on medication	4 (57.1)	7 (50.0)	—
Hypertension	5 (71.4)	10 (71.4)	1
BMI	22.9 ± 2.4	22.9 ± 3.3	0.852
AIS LAA	5 (71.4)	3 (21.4)	0.056
SVO	2 (28.6)	11 (78.6)	
AS score	5.3 ± 3.7	2.1 ± 2.0	0.019
Anterior c	2.6 ± 1.8	0.9 ± 1.3	0.035
Posterior c	2.3 ± 2.9	1.1 ± 0.9	0.588
T-CUS	2.0 ± 2.3	0.6 ± 0.9	0.133
T-EUS	4.0 ± 1.0	1.8 ± 1.1	0.031
T-ABI	1.9 ± 0.4	2.1 ± 0.3	0.459

The differences between the two groups were evaluated statistically by χ^2 or Mann-Whitney's U tests.

Abbreviations: AIS – acute ischemic stroke; SVO – small-vessel occlusion; LAA – large-artery atherosclerosis; AS – atherosclerosis score; anterior c – anterior circulation; posterior c – posterior circulation; T-CUS – total carotid ultrasonic score; T-EUS – total extremities ultrasonic score; T-ABI – total ankle-brachial index.

Table 4. The comparison of laboratory data between the patients of acute ischemic stroke with nephrotic syndrome due to diabetic nephropathy (NS group) and acute ischemic stroke with diabetes mellitus without nephrotic syndrome (Control group)

	NS group	Control group	P
Albumin	2.7 ± 0.5	4.2 ± 0.4	<0.001
Total cholesterol	321.7 ± 42.5	211.7 ± 39.4	0.001
LDL-cholesterol	214.4 ± 40.5	132.6 ± 34.2	0.001
HDL-cholesterol	51.3 ± 17.4	49.4 ± 9.7	0.94
Triglyceride	229.0 ± 67.4	189.6 ± 203.1	0.037
Creatinin	2.1 ± 1.6	0.9 ± 0.3	0.009
eGFR	37.7 ± 23.3	71.5 ± 22.6	0.011
Hematocrit	35.7 ± 5.5	43.0 ± 5.1	0.006
Platelet	27.8 ± 2.7	22.4 ± 5.8	0.048
PT	0.9 ± 0.7	0.9 ± 0.0	0.55
APTT	28.9 ± 3.9	28.8 ± 3.1	0.933
Fibrinogen	385.4 ± 104.5	275.0 ± 57.9	0.019
D-dimer	1.8 ± 1.0	0.7 ± 0.4	0.01

The difference between two groups was statistically estimated by the method of χ^2 test or Mann-Whitney's U-test.

Abbreviations: LDL – low density lipoprotein; HDL – high density lipoprotein; eGFR – estimated glomerular filtration rate.

4. CEREBRAL VENOUS SINUS THROMBOSIS (CVT) ASSOCIATED WITH NS

1) CVT Patients (Table 5)

Case 1

The patient was a 46-year-old man. He had headache and vomiting the day after he drank heavily. Contrast brain computed tomography (CT) and MRI revealed a defect in the transverse sinus, straight sinus, and superior sagittal sinus. His blood was hemoconcentrated, and blood test results indicated high D-dimer and fibrinogen levels and decrease of antithrombin III.

Case 2

The patient was an 89-year-old woman. She suffered from ischemic colitis. She developed left hemiplegia and headache after having had diarrhea for a month. Brain CT revealed hematoma in the subcortical region of the right frontal lobe and a high signal in the straight sinus. The superior sagittal sinus showed high-signal intensity on T1-weighted MRI and mild high-signal intensity on T2-weighted MRI. High D-dimer and fibrinogen levels were detected in the blood.

We experienced 25 cases of CVT over a period of nine years from April 2004 to August 2013. Of these cases, two had a complication of NS. The incidence of NS in CVT patients

was 8.0%. Over the same period, we had 10 cases of AIS-associated NS, which is five times that of CVT associated with NS.

Table 5. The patients with cerebral venous sinus thrombosis

				MRI/CT							
Case	Age	Sex	Type	Location of occlusion	Alb	TC	UP	day	E	DM	NS type
1	46	M	CVT	SSS, LS, straight S	2.4	364	4	3.5	+	—	minimal change
2	89	F	CVT	SSS, straight S	1.9	326	4	>3.5	+++	—	unknown

Abbreviations: TC – total cholesterol; Alb – albumin; UP – urinary protein; E – edema; DM – diabetes mellitus; NS – nephrotic syndrome; CVT – cerebral venous sinus thrombosis; SSS – superior sagittal sinus; LS – lateral sinus (transverse sinus).

Table 6. The adult cases of cerebral sinus thrombosis with nephrotic syndrome

Authors	year	Cases	Age/sex	Disease	Clinical pictures	Site of thrombosis	Rx	Outcome
Barthélémy	1980	1	50/M	MCD	H, papilledema	SSS, LS	AC	CR
Tovi	1988	2	46/M	n.a.	SVC syndrome	LS		CR
Levine	1989	3	32/M	MPG	H, papilledema, IV pa/sy	SSS,	AC	CR
Utunomiya	1994	4	29/M	MCNS	H, hemi-p	SSS, LS	AC	?
Burns	1995	5	20/M	MCNS	S, personality changes	SSS		Fetal
		6	35/M	MCNS	H, III palsy, hemi-p, aphagia	SS	AC	PR
Laversuch	1995	7	42/F	MGN	H, S, confusion, hemi-p	LS	AC	CR
Urch	1996	8	41/F	MCD	H, aphagia, hemi-p	SSS	AC	CR
Akatsu	1997	9	65/F	MCD	H, hemi-p	SSS		CR
Koch	1997	10	23/F	n.a.	H, visual change	SSS	AC	CR
Hirata	1999	11	46/M	MCNS	H, papilledema	SSS, LS	AC	CR
Philips	1999	12	29/M	na	S, VI palsy	SSS, LS, SS, IJV	Lysis	CR
Sung	1999	13	45/M	CTIN	H, hemi-p	SSS		CR
Nishi	2006	14	79/F	AMD	S	LS, SS		Fetal
Komada	2007	1	29/M	MCNS	H,	SSS	AC	CR
Ozkart	2011	16	56/M	MGN	H, S	LS, Straight S	AC	CR
Present cases		17	46/M	MCD	H	SSS, LS, straight S	AC	CR
		18	89/F	n.a.	H, hemi-p	SSS, straight S	AC	Fetal

Abbreviations: Rx – treatment; MCD – minimum change disease; MCNS – minimum change nephritic syndrome; MPG – mesangial proliferative glomerulonephritis; MGN – membranous glomerulonephritis; CTIN – chronic tubulointestinal nephritis; AMD – amyloidosis; n.a. – not available; H – Headache; S – seizure; hemi-p – hemiplegia; SSS – superior sagittal sinus; LS – lateral sinus (transverse sinus); SS – sigmoid sinus; IJV – internal jugular vein; AC – anticoagulation therapy; lysis – endovascular thrombolysis; CR – complete remission.

2) Literature Review of CVT Patients with NS

We reviewed the literatures of CVT patients with NS since 1980. The 54 cases under 20 years old were reported from Japan (20 cases) and other countries (34 cases). The 29 adult cases over 20 years old, including our two cases, were reported from Japan (10 cases) and other countries (19 cases). Table 6 shows the clinical characteristics of 18 cases (12 male and 6 female), omitting those cases for which only an abstract was available [28-42]. The average age of the cases was 44.6 ± 18.5 years (20-89 years) and minimal change disorder was detected as the cause of NS in eight cases. The clinical symptoms included headache in 14 cases (77.8%), convulsion in five cases (27.8%), motor hemiparesis in seven cases (38.9%), cranial palsies in three cases (16.7%) and aphagia in two cases (11.1%). Occluded sinuses were observed in superior sagittal sinus or transverse sinus in 13 cases (72.2%).

NS had already been diagnosed in 13 cases before the occurrence of CVT, but in five cases was not diagnosed until the occurrence of CVT [29, 36, 39-41]. The patients showed the abnormal hypercoagulation, including decreases of antithrombin III in six cases, decrease of protein S in two cases, increased fibrinogen in 10 cases, positive lupus anticoagulant in three cases, positive antinuclear antibody in two cases, factor V Leiden mutation with hyperhomocysteinemia in one case, hemoconcentration in two cases, hormone replacement therapy with estrogen in one case, preeclampsia in one case, SLE in one case, rheumatoid arthritis (RA) in one case, ischemic colitis with diarrhea in one case, and heavy drinking in one case (Table 7).

5. INTRACEREBRAL HEMORRHAGE (ICH) ASSOCIATED WITH NS

1) ICH Patients

Case 1

A 51-year-old woman was admitted to our hospital with headache and vomiting. She had right hemiparesis and speech disturbance. At age 46, she had suffered from DM, but did not take any medications. At age 47, she had double vision due to right abducent nerve palsy, and was diagnosed as having a right carotid-cavernous fistula (CCF). Endovascular coiling embolization was performed. On admission, she had slight unconsciousness (JCS I-2), dysarthria, and right side hemiparesis. NIHSS was 5 points. Her blood pressure was 170/92 mmHg. MRI showed the subcortical hemorrhage (7.1 ml) in the left temporal lobe. No abnormal vessel or microbleeds were detected in MRA and T2* weighted MRI. The conservative treatment was performed, but the peripheral edema and pleural effusion were not improved. On day 12, she was transferred to a specialist nephrology hospital, scoring 3 on the modified Rankin scale (mRS).

Case 2

A 63-year-old woman was admitted to our hospital because of gait disturbance. She had left hemiparesis and speech disturbance. At age 56, she had suffered from intracerebral hemorrhage (left putamen), and was under the treatment of hypertension and DM for several

years. On admission, she had slight unconsciousness (JCS I-2), dysarthria, and left side hemiparesis. She also had slight residual of right hemiparesis. Bilateral pyramidal tract signs were observed. NIHSS was 6 points and her blood pressure 220/120 mmHg. A peripheral edema was observed in her lower extremities. MRI showed a hemorrhage (2.2 ml) in the right putamen, and an old lesion (slit) in the left putamen. No microbleeds were detected in T2* weighted MRI. On day 16, the patient was transferred to a rehabilitation hospital on mRS 2 following treatment on the acute stage of putaminal hemorrhage.

Case 3

A 72-year-old man was admitted to our hospital because of generalized convulsion. He had recurrent episodes of lobar hemorrhage (subcortical hemorrhage). He was bed-ridden after 10 episodes over a period of several years. He had vascular dementia (MMSE; 7/30 points). On admission, he had quadriplegia and bulbar palsy, showing bilateral pyramidal signs. He showed dyspnea due to pneumonia and marked peripheral edema in his extremities. His blood pressure was 104/70 mmHg. He had no DM. MRI showed a subcortical hemorrhage in right frontal lobe, and many microbleeds in bilateral frontal, temporal, parietal and occipital lobes, some of which presented as accumulations or clusters. MRA showed multiple stenosis of intracerebral arteries. The patient was diagnosed with cerebral amyloid angiopathy. He was treated by methyl prednisolone, but the excretion of urinary protein was not improved. Two months later, he was transferred to a specialist nephrology hospital on mRS 5.

Case 4

A 76-year-old man was admitted to our hospital because of vertigo. At age 52, he had a subarachnoidal hemorrhage and underwent an operation for a ruptured aneurysm. For several years, he received treatment for hypertension and DM, which were not well controlled. On admission, he had cerebellar ataxia and dysarthria, but no meningeal irritation or pyramidal signs. NIHSS was 3 points. His blood pressure was 200/80 mmHg. He showed peripheral edema in his lower extremities. MRI showed the hemorrhage (3.1 ml) in the deep part of left cerebellar hemisphere with little extravasation in ventricles. An old infarct lesion in the right temporal lobe and ventricular dilatation were recognized. On day 26, he was transferred to a rehabilitation hospital on mRS 5 after treatment on the acute stage of the cerebellar hemorrhage.

Tables 8 and 9 show the clinical characteristics of, and laboratory findings for the patients with ICH with NS. Increases of HbA1c, fibrinogen, d-dimer and creatinine were found in three cases, two cases, two cases and three cases, respectively. Serum level of antithrombin III was normal in two cases examined. ABI was abnormal in one case, and ultrasonography of carotid artery detected mild to moderate stenosis of internal carotid artery in two cases.

The basic diseases underlying NS were suggested as amyloid nephropathy in Case 3 and DMN in Cases 1, 2 and 4. The incidence of ICH associated with NS was 0.18% of total stroke or 0.036% of ICH.

Table 7. The hypercoagulability factors in the cases of cerebral sinus thrombosis with nephrotic syndrome

Cases	Age/sex	D-dimer‡	AT III	Procin S	Fbg	LA	ANA	others
1	50/M							
2	46/M				H			otitis media
3	32/M	N	N			+	—	
4	29/M	H	44.00%		H	—	—	onset on the second day of steroid pulse therapy
5	20/M		L (59 u/dl)*				—	
6	35/M		N	N	H			
7	42/F		N	17%		+	+	SLE
8	41/F							
9	65/F		33%	N		—	—	hormone (estrogen) therapy
10	23/F		N	N	H	—	—	preeclampsia, onset on the 8 th day after labor
11	46/M	H	68%		H	—	—	
12	29/M					+		endovascular thrombolysis
13	45/M		N	71%	N	—	+	
14	79/F	H	N		H			RA for 20 years
15	29/M	H	L (7 mg/dl)**		H		—	
16	56/M	H	N	N	H	—	—	factor V Leiden: mutation+, Hcy-mia
17	46/M	H	80%	N	H	—	—	HC, heavy drinking
18	89/F	H			H			ischemic colitis, diarrhea

Abbreviations: Fbg – fibrinogen; LA – lupus anticoagulant; ANA – antinuclear antibody; H – high; L – low, N – normal; Hcy-mia – homocystinemia; ‡: D-dimer or FDP (fibrin degradation products) were measured.

* normal range 80-140.

** Normal range was not described.

Table 8. Clinical characteristics in the patients of intracerebral hemorrhage with nephrotic syndrome

Case	Age	Sex	Type	MRI findings	Alb	TC	UP, g/day		E	NS type	HT	CAD	C-US	ABI	NIHSS	mRS	others
													rt/lt	rt/lt	at ad.	at dis.	
1	51	F	ICH	LB, temporal Lobe	2.3	363	4+	5.7	++	DMN s/p	+	—	/	/	5	3	CCF (47y)
2	63	F	ICH	Rt. putamen	2.9	348	4+	/	+	DMN s/p	+	—	/	1.15/ 1.19	6	2	ICH (61y, lt. putamen)
3	72	M	ICH	LB, multi stenosis	2.0	274	4+	/	++	AMN	—	+	0/1	1.22/ 0.85	/	5	recurrent LB
4	76	M	ICH	Cerebellum	2.7	282	4+	9.5	+	DMN s/p	+	—	¼	/	3	5	SAH (52y), NPH

Abbreviations: ICH – intracerebral hemorrhage; LB – lobar bleeding (subcortical hemorrhage); TC – total cholesterol; Alb – albumin; UP – urinary protein; E – edema; NS – nephrotic syndrome; CAD – coronary artery disease; C-US – ultrasonography of carotid artery; ABI – ankle-brachial index; NIHSS – NIH stroke scale; mRS – modified Rankin scale; CCF – carotid-cavernous fistula; DMN – diabetic nephropathy; AMN – amyloido nephropathy; s/p – suspected; ad. – admission; dis – discharge.

Table 9. Laboratory findings in the patients of intracerebral hemorrhage with nephrotic syndrome

Case	Cr	eGFR	DM, years		HbA1c	TG	HDL	LDL	Hct	Plt	PT	aPTT	Fbg	Ddimer	AT III
1	2.7	15.6	+	5	9.0	219	44	275	34.1	33.2	0.97	36.2	450	2.3	96
2	1.8	22.9	+	7	8.2	192	101	208	35.9	31.4	0.9	23.3	277	0.6	/
3	0.5	121.4	—	/	4.8	101	117	114	41.5	28.2	/	/	/	/	/
4	1.3	42.0	+	5	6.5	109	73	187	49.8	25	0.83	27.4	378	1.2	83

Abbreviations: Cr – creatinine; eGFR – estimated glomerular filtration rate; DM – diabetes mellitus; year – years of disease duration; TG: triglyceride – HDL – high density lipoprotein; LDL – low density lipoprotein; Hct – hematocrit; Plt – platelet counts; PT – prothrombin time; aPTT – activated partial thromboplastin time; Fbg – fibrinogen; AT – antithrombin.

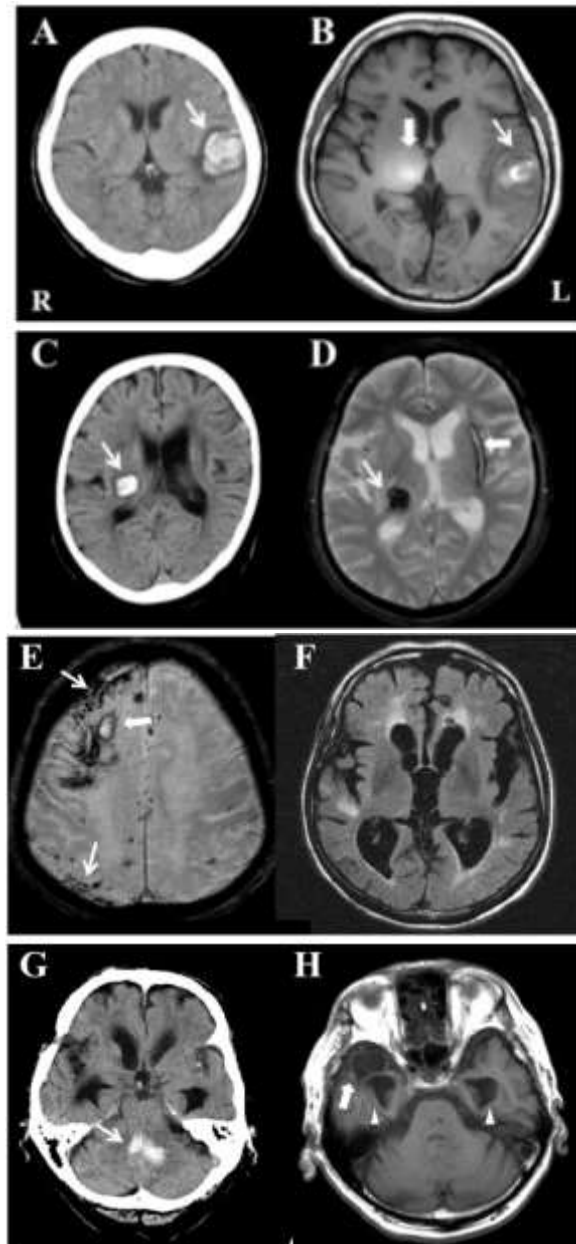


Figure 3. CT and MRI of the patients with intracerebral hemorrhage Case 1 (A, B); The arrows indicated subcortical hemorrhage (7.1 ml) in the left temporal lobe. The broad arrow was the artifact due to the coils. A, Brain CT, B; T1 weighted image, 1.5T, axial, TR; 525ms, TE; 12 ms . Case 2 (C, D); The arrows indicated the hemorrhage (2.2 ml) in the right putamen, and the broad arrow indicated the old hemorrhagic lesion of the left putamen. C, Brain CT, D; T2* weighted image, 1.5T, axial, TR; 660 ms, TE; 25 ms. Case 3 (E, F); Broad arrow indicated subcortical hemorrhage in the right frontal lobe. Many micro bleeds were showed, and some (arrows) were clustered. Brain atrophy with ventricular dilatation and periventricular high signals were also elucidated. E; T2* weighted image, 1.5T, axial, TR; 814 ms, TE; 24.6 ms. F; T2 weighted image, 1.5T, axial, TR; 10000 ms, TE; 120 ms. Case 4 (G, H); The hemorrhage (arrow) in the deep part of cerebellar hemisphere, the old infarct (broad arrow) in the polar head of right temporal lobe and the ventricular dilatation (arrow heads) were shown. G; Brain CT, H; T1 weighted image, 1.5T, axial, TR; 530 ms, TE; 12 ms.

DISCUSSION

Patients with NS have a high incidence (21 to 51% of patients) of venous thrombosis, particularly deep vein and renal vein thrombosis, and pulmonary emboli, especially in younger patients under 20 [43-48], and CVT has also (rarely) been reported [2]. On the other hand, the relative risk of arterial thrombosis is low (1.0 to 5.5%), compared to that of venous thrombosis [3, 49, 50]. In 2014, Sasaki et al. reported an additional case of AIS with NS, and reviewed 21 prior cases reported in 19 literatures [4]. However, the incidence and clinical characteristics of AIS patients have remained unclear [5-10]. We reported six LAA patients, three SVO patients and one CE patient, and elucidated the following. The incidence of AIS associated with NS was 0.09% of total stroke or 0.12% of AIS, and five times that of CVT in the adult patients with NS. The patients had severe stenosis and/or occlusion of intracranial cerebral arteries, especially in the cerebral anterior circulation, and also demonstrated atherosclerosis of the internal carotid and lower extremity arteries. The patients had high serum fibrinogen and D-dimer levels, suggesting that AIS patients with NS have a greater degree of hypercoagulability than AIS patients without NS. The pathogenesis of NS was from DMN in eight cases (80%), amyloid nephropathy in one case, and MPGN in one case. These results suggest that the AIS patients with NS, especially from DMN, had marked general arteriosclerosis of the body, which might be a clinical characteristic peculiar to Japanese patients [24].

Mahmoodi et al. reported that annual incidences of venous and arterial thromboembolism in NS patients were 1.02% and 1.48%, respectively – eight times higher than in general population from a retrospective cohort study. They also reported that multiple classic risk factors for atherosclerosis are associated with arterial thromboembolism in NS patients, including sex, age, hypertension, DM, smoking, prior arterial thromboembolism, and eGFR. The annual incidence of DMN patients was 7.43, which was the highest incidence among all types of nephropathy [3].

Sasaki et al. reported that histopathology of NS in the 22 AIS patients previously reported from several countries was membranous nephropathy in five cases (22.7%), minimal change in four cases (18.2%), MPGN in three cases (13.6%), focal segmental sclerosis in two cases (9.0%), Ig A nephropathy, undetermined pathology in six cases (27.2%), and DMN in only one case (4.5%) [4]. In our study, DMN was clinically apparent for the pathogenesis of NS in 80% of patients. The occurrence of non-diabetic renal disease (NDRD) in DMN patients has been increasingly recognized in recent years. Zhuo et al. reviewed the 13 literatures from the period between 1983 and 2012, and reported the prevalence of DMN complicating NDRD; the common histological diagnoses were tubule-interstitial nephritis (22.7%) and IgA nephropathy (14.1%) [51]. The complication of NDRD notwithstanding, DMN could be the primary cause of the pathogenesis of NS in 80% of our patients. The patients with NS had the combined risks from the basic, disease-induced NS, as well as the hypercoagulation state arising as a result of NS. It is well known that DM and hyperlipidemia are strong risk factors for AIS, but it is possible that the existence of NS may be another risk factor. DM may be the strongest and most important risk factor for AIS associated with NS, especially in Japanese patients.

CVT commonly occurs in young adults under 40 years old, and is around three times more common in females than in males. Its incidence is about 0.5-1.0% among all types of

stroke. There are many risk factors of CVT, one of which is NS [2, 11, 12, 52-54]. Twenty-five patients (aged 59.8 ± 18.6 years; 20 men and five women) were admitted to our center during a nine-year period between April 2004 and August 2013 [55]. The incidence of CVT in our study was 0.22% of all kinds of strokes or 0.30% of ischemic infarctions, which may be lower than previously reported. The patients with CVT in our study had, as risk factors, oral contraceptive use (two patients), deficiency of protein S (three patients) and antithrombin (two patients), nephrotic syndrome (two patients), iron deficiency anemia (three patients), colon cancer (one patient), and multiple myeloma in remission (one patient). Furthermore, hyperhomocysteinemia was identified in nine (56.3%) of 16 patients tested (Table 10). Our results show that the CVT patients in Japan may be older and include fewer females than CVT patients in Western countries [55]. The most common risk factor in women has been reported to be oral contraceptive use [2, 11, 12, 14, 15, 16]. Oral contraceptive use has been reported to be less prevalent as a cause of CVT in Japan than in Western countries [56]. This may be due to few young female CVT patients using oral contraceptives in Japan.

NS is one of the risk factors for CVT [28, 53, 54]. A multinational (21 countries), multicenter (89 centers) prospective observational study entitled 'The International Study on Cerebral Vein and Dural Sinus Thrombosis' (ISCVT) [52, 57-60] examined 624 cases of CVT. ISCVT and other studies reported that the prevalence of NS was 0.6-2% [12, 52, 54, 61]. In our study, NS was recognized as a risk factor for CVT in two out of 25 cases (8.0%) [55]. Our study was not performed in multiple centers, but in only one center. In the future, a nationwide prospective study needs to be performed in order to clarify the characteristics of CVT in more detail, especially its risk factors, in Japanese patients of all ages. Patients with NS have a thrombotic tendency; both venous thrombosis and arterial thrombosis may occur. In the literature, the number of published cases of CVT was tenfold that of cerebral artery thrombosis as a complication of NS in individuals aged <20 years. In adults, however, the number of cases of CVT reported was twice that of cerebral artery thrombosis cases. Venous thrombosis is likely to occur in young cases with NS, and both venous and arterial thromboses are likely to occur in adult cases [25]. NS shows a thrombotic tendency, but the onset of CVT may develop as a result of some additional thrombotic factor, such as hemoconcentration after heavy drinking, diarrhea, or vomiting. Lifestyle management alongside the conventional treatment of NS is important.

It is well known that hemorrhagic lesions possibly occur as an associated complication of CVT [2]. However, the clinical details of ICH-associated NS have not been reported [62]. Patients with NS have a thrombotic tendency, which, as described above, is a risk factor for both venous thrombosis and arterial thrombosis. When we retrospectively analyzed the hospitalized patients, however, we not only found the 10 AIS and 2 CVT patients with NS, but also, unexpectedly, four ICH patients with NS [26]. The incidence of ICH associated with NS was 0.18% of total stroke or 0.036% of ICH. The basic disorders associated with NS were DMN and amyloidosis in three cases and one case, respectively. These patients had strong risk factors for ICH, such as hypertension, vascular vulnerability with atherosclerosis or amyloidosis, with change of bloodstream after operation, suggesting that they had overcome the risk of thrombophilia (Figure 3). NS essentially associates with a risk for venous or arterial thrombosis. Attention should also be paid to NS as a risk factor for ICH.

The main cause of NS in children is minimal change disease (MCD). On the other hand, approximately 30% of adults with NS have a systemic disease, such as DM, amyloidosis, or SLE. The remaining cases are usually due to primary disorders including MCD, focal

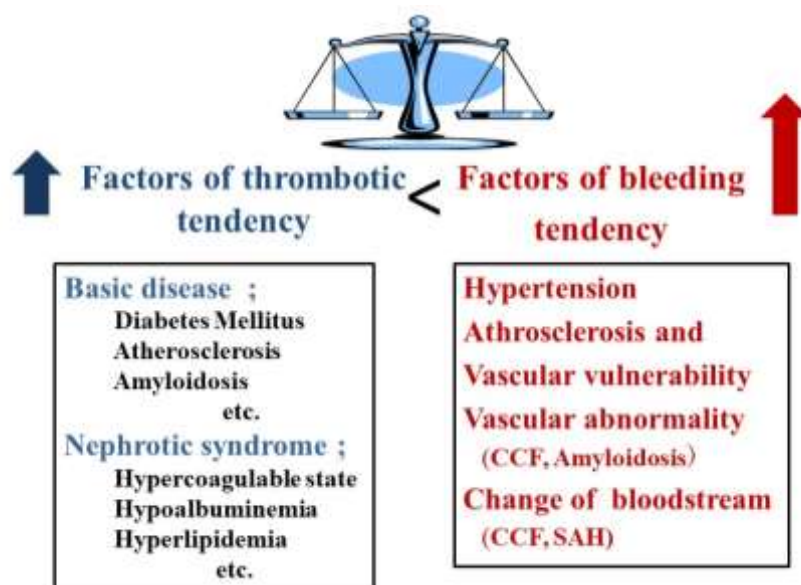
segmental glomerulosclerosis (FSGS), and membranous nephropathy. The frequency of the different forms of nephropathy underlying NS in adults was evaluated in renal biopsy registries of several counties [63-70]. Among patients between the ages of 15 and 65, the most common causes of NS were membranous nephropathy, MCD, lupus nephritis, FSGS, MPGN, amyloidosis, and IgA nephropathy. A similar distribution was observed among elderly individuals (aged above 65 years), except for an increased prevalence of amyloidosis and a decreased prevalence of lupus. The frequency of the DMN in these registries was ~1-5% [63-70].

Table 10. The risk factors and etiology in the patients with cerebral venous sinus thrombosis

Patients	n = 25	%
Sex (male)	20	80
Age		
Male	59.8 ± 17.6	
Female	59.6 ± 24.4	
Lab. findings and risk factors		
d-dimer n = 20	20	100
Protein C deficiency n = 15	0	0
Protein S deficiency n = 15	3	20
Antithrombin deficiency n = 14	2	14.3
ANA n = 14	0	0
Anti-phospholipid syndrome n = 15	0	0
CRP n = 21	19	90.5
Fibrinogen n = 18	14	77.8
Anemia (fe def.) n = 25	3	12
Homocysteine n = 16	9	56.3
Oral contraceptives (female n = 5)	2	8 (40)
Nephrotic syndrome	2	8
Malignancy	1	4
Others (associated)		
Multiple myeloma	1	
Multiple sclerosis	1	
Neurofibromatosis 1	1	
Epilepsy	1	

In Japan, the frequency of nephropathy underlying NS was pathologically evaluated in the Japan Renal Biopsy Registry (J-RBR) of 2,751 patients. Among patients between 20 and 65 years of age, the most common causes of NS were membranous nephropathy (18%), minimal change disease (25%), DMN (12%), lupus nephritis (10%), FSGS (7%), MPGN (2%), amyloidosis (2%), and IgA nephropathy (7%). Among the elderly individuals (age greater than 65 years), the most common causes of NS were membranous nephropathy (32%), MCD (13%), DMN (10%), lupus nephritis (2%), FSGS (6%), MPGN (4%), amyloidosis (8%), and IgA nephropathy (4%). The most remarkable characteristic form underlying NS in the J-RBR evaluation was the higher frequency of DMN than those in the other registries [71,

72]. Furthermore, it has recently been reported that 41.4% of 614 patients with pathologically confirmed DMN registered in J-RBR were in a clinically nephrotic state [73]. In our stroke registry, DMN was the main disorder underlying NS in both AIS and ICH associated with NS (Figure 4). Long-term DM could damage the arterial vessels so as to precipitate the occurrence of both diseases. On the other hand, DM cannot significantly damage the veins, indicating that the thrombotic tendency due to NS may be the main player in causing CVT. NS tends to be associated with a risk factor for AIS and CVT, and attention must also be paid to ICH associated with NS in cases of stroke.



CCF: carotid-cavernous fistula.

Figure 4. The factors of thrombotic tendency and the factors of bleeding tendency in the patients with intracerebral hemorrhage associated with nephrotic syndrome.

AIS		CVT		ICH	
NS	0.12 %	8.0 %		0.036%	
n=10		n=2		n=4	
DMN 8		MCD 1		DMN 3	
AMD 1		nd 1		AMD 1	
MPGN 1					

AMD – amyloido nephropathy; MPGN – membranoproliferative glomerulonephritis; MCD – minimal change disease.

Figure 5. Nephrotic syndrome (NS) and diabetic nephropathy (DMN) in the patients with acute ischemic stroke (AIS), cerebral venous sinus thrombosis (CVT) and intracerebral hemorrhage (ICH).

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Chapter 90

POST-STROKE DYSPHAGIA

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ABSTRACT

Dysphagia is a common consequence after stroke, presenting in up to 50% of all acute stroke patients, with increased morbidity and mortality mainly because it dramatically increases the risk of aspiration pneumonia, and because it leads to malnutrition and dehydration as well.

It has been reported that the incidence of dysphagia ranges from 25% to 81% depending on the timing of the assessment, diagnostic methods and criteria.

The nervous system affected by a stroke damage is thought to reserve a variable ability to somehow reorganize damaged pathways and structures, allowing an effective recovery from swallowing impairment.

Notwithstanding, about half of stroke patients remain dysphagic after seven days from symptoms onset, and approximately 13-15% of patients has persistent swallowing dysfunction after six months, with a dramatic impact on patients' outcome in terms of increased likelihood of residential placement and estimated lifetime costs in stroke survivor.

To date, it is unclear why some stroke patients develop a persistent swallowing impairment after stroke or, in other words, if the failure in recovery from dysphagia is due to a stroke involving the dominant swallowing hemisphere, rather than a swallowing key region, or even to any specific clinical or neuroradiological stroke factor.

In this view, stroke severity upon admission, assessed using NIHSS, is an important clinical predictor of post-stroke dysphagia and of a persistent pattern of dysphagia as well.

Regarding the site of the stroke lesion, it is widely accepted that swallowing function is provided by a complex neural network bilaterally involving the perirolandic sensorimotor cortex which, together with other cortical structures, projects and receives

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afferences to the brainstem' Central Pattern Generator, through the basal ganglia, the thalamus and the periventricular white matter as well. Despite several cortical areas have been described as related with swallowing impairment, a definite role in causing dysphagia has been not completely understood for all of them so far.

Interestingly, recent studies reported that the disruption of cortical-cortical and cortical-subcortical white matter connections seems to increase the risk of dysphagia and aspiration pneumonia by lowering the threshold of input to the medullary swallowing center.

Thus, dysphagia may result from the combined effects of an acute focal damage and a preexisting impairment of the subcortical white matter connecting pathways. In other words, we might think of dysphagia as a kind of deficit in executive functions, which we know to be particularly associated with leukoaraiosis and cognitive disorders, and of course age.

Keywords: leukoaraiosis, dysphagia, stroke

INTRODUCTION

Dysphagia is a common consequence after stroke, presenting in up to 50% of all acute stroke patients. It has been reported that the incidence of dysphagia ranges from 25% to 81% depending on the timing of the assessment, diagnostic methods and criteria.

The clinical and scientific relevance of post-stroke dysphagia is demonstrated by its high incidence and negative effect on clinical outcome, with increased morbidity and mortality mainly because it dramatically increases the risk of aspiration and pneumonia [1, 3-6].

In this view, pulmonary infections represent one of the most common and fearsome complications in stroke patients: SPREAD guidelines report that, along with urinary infections, it reaches a cumulative incidence of about 30%, hence far exceeding that of other internal medicine complications [7].

Aspiration pneumonia is also associated with a relevant worsening of stroke outcome: a recent multicenter observational study, carried out on 8,250 stroke patients, confirmed that lung infections in patients with stroke are associated with worsening of mortality both at 30 days and at one year after the acute phase of stroke, with an increased length of stay, a greater disability at discharge, and an increased percentage of stroke patients sent in long-term care [4].

In the literature there are also conflicting reports on the consequences of the so called "silent aspiration," which is the aspiration of swallowed material without any clinical manifestations, and thereby underdiagnosed. It is estimated to be prevalent in 15-39% of patients with subacute stroke and in 2-25% of unselected patients with acute stroke, with a relevant increase in relative risk of pneumonia and a poorer clinical outcomes for silently aspirating patients compared with overt/non-aspirators [8, 9].

This data, as well as the strong evidence that dysphagia significantly contributes to malnutrition and dehydration regardless of aspiration [10], highlight the dysphagia's dramatic impact on patients outcome, with increased likelihood of residential placement and significantly increases estimated lifetime costs in stroke survivors [11].

NEURAL CONTROL OF SWALLOWING

It is now widely recognized that swallowing process is mediated by neural pathways that involve cortical input reaching the brainstem by means of fibers, such as the corticobulbar tract, which connect cortical areas that control swallowing with bulbar nuclei that mediate the final mechanism; however, the role of the different cortical and subcortical areas in causing dysphagia is still not entirely clear.

Several studies have dealt with identifying those structures involved in the process of swallowing.

In the past, researchers have mainly focused their attention on specific regions of the brainstem, in order to evaluate the action of interneurons involved into the oral and pharyngeal swallowing.

The focus then shifted on the observation of the interaction between different cortical areas and brainstem nuclei to control swallowing [12-15].

At a subcortical level, a consistent peristaltic contraction is guaranteed by a number of interneurons located in the reticular formation, in the nucleus of the solitary tract and in the nucleus ambiguus as well, all regions which constitute the swallowing core regulator of the brainstem. These centers are responsible for the integration of information coming from the oropharynx, esophagus and from supranuclear structures and are classically described as “central pattern generator” (CPG).

CPG is mainly entrusted with the control of both timing and synchronism of swallowing process. In any case, the brainstem is a part of a complex cortical-subcortical network which involves a sensory system, motor system, and white matter pathways. In this point of view, the majority of the recent studies highlight that the integrity of these connections appears to be fundamental for an effective coordination of swallowing to be achieved [16-18].

Brainstem and Peripheral Control

Since early researches carried on by Car and coworkers [19, 20], the brainstem has been described as responsible for the complex sequences and execution of the neuromuscular events involved in swallowing, by means of the compact clustering of bilateral cranial nerves nuclei and interneuronal connections as well [21]. The very first evidence of this role came from a study of decerebrated animals [22]: it has been demonstrated an elicitation of pharyngeal and esophageal swallowing obtained by stimulating this area in the absence of cortical input.

It is now widely accepted that brainstem structures provide the basic motor plan for pharyngeal swallowing, being also involved in the coordinative interactions between swallowing and respiration [23].

As mentioned above, the swallowing core regulator of the brainstem is represented by the Central Pattern Generator (CPG), a structure located into the rostral and ventrolateral portion of the brainstem. From an anatomical and functional point of view, the CPG has been classically divided in two bilateral region [24]: a dorsal region, which includes the nucleus of the solitary tract (NTS) with the adjacent reticular formation, and a ventral region, represented by the area surrounding and including the nucleus ambiguus (NA).

Since NTS represents the primary sensory nucleus for the VII, IX and X cranial nerve, the dorsal region is thought to represent the initial neuronal entrance for input that modulates swallowing: sensitive afferent fibers originate from mucosal chemoreceptors, mechanoreceptors and thermoreceptors of the oral cavity, pharynx and larynx; these fibers are involved in both the initiation and repeated activation of pharyngeal phase of swallowing, and are essential for the protection of the airway by the phenomenon of aspiration.

Muscle spindle receptors located in the pharyngeal and esophageal swallowing muscle, are otherwise considered as a trigger for those interneurons that modify motor output to muscle [21].

Otherwise, CPR ventral region is involved in sending out neural commands that control pharyngeal, laryngeal, and esophageal muscles. This motor task is carried out by means of NA, which is the primary motor nucleus for IX, X and XI cranial nerves, and has several interconnections with other medullary motor nuclei, such as those of V, VII and XII cranial nerves.

NA also contains axons projected to contralateral brainstem regions, suggesting that the ventral region has also a role in the integration of sensory information; this suggestion is otherwise confirmed by the fact that the ventral efferent medullary region receives direct input from the dorsal afferent medullary one. Hence, it could be argued that sensory information entering the brainstem via the NTS is integrated by the NA into a swallowing motor plan, and then transmitted to the motor nucleus for execution [21].

Finally, the interneuronal networks localized in CPG integrates sensory information by modulating afferent and efferent pathways activation which reciprocally connects the brainstem to the higher centers. This circuit is thought to represent the way how motor circuits are modulated by sensory feedback pathways, depending on bolus characteristics, head position and on muscular activity.

Higher Nervous System Control

While the basic motor plan for pharyngeal swallowing is provided by brainstem structures, the start of swallowing is a voluntary act which depends on the integrity of several cortical areas.

It has been suggested that the programming of the swallowing process firstly occurs in non-primary motor areas (i.e., mesial premotor cortex), secondly involving the primary sensorimotor cortex, and finally reaching the secondary areas.

Several studies [25-29] have identified numerous areas of interest who have a significant role in swallowing, such as the premotor cortex, the primary sensorimotor cortex, the insula, as well as some limbic areas. However, although these foci have been consistently identified in many of these studies, the exact width and functional relevance of the entire swallowing network have not been entirely clarified so far.

Discrepancies between the studies probably reflect methodological differences: the main imaging techniques used to investigate the neural basis of swallowing (i.e., functional Magnetic Resonance Imaging (fMRI), Positron Emission Tomography (PET) and Magnetoencephalography (MEG)) actually offer a representation of different aspects of brain activity. FMRI detects changes in local cerebral oxygenation, closely linked to the underlying neuronal activity; PET uses radioactive markers to study local changes in neurophysiological

parameters as cerebral perfusion and glucose consumption; finally MEG measure small magnetic fields arising from neural.

Among these, fMRI is the most widely adopted technique, mainly because of its safety and its spatial resolution of about 4 mm; hence according to some Authors it could be used as an auxiliary technique to the conventional MRI to investigate the dysphagia following brain injuries. However even fMRI studies are heterogeneous with regard to the task investigated (voluntary or reflex swallowing, number of swallows, etc.).

An interesting meta-analysis [30] based on the activation likelihood estimation (ALE), has compared some of these studies in order to better identify brain areas involved in both water and saliva voluntary swallowing. The ALE is a quantitative voxel-wise meta-analysis technique which estimates the probability of brain activation in a given voxel, by analyzing the results of several studies expressed as standard space coordinates of activation maxima. The meta-analysis of brain activation showed that the significant activation clusters seen during water swallowing were partly different from those seen during saliva swallow. Authors also performed a between condition meta-analysis showing that, despite a partial overlap in the network involved in water and saliva swallowing, the first is strongly associated with right inferior parietal activation, while the latter more strongly involves premotor areas. This datum suggests that water swallowing strongly activates those cortical areas involved in sensory processing of intraoral water stimulation, while saliva swallowing massively involves premotor areas, which are crucial for initiation and control of swallowing sequence movements.

To have an overview of the several cortical and subcortical regions involved in swallowing, Table 1 report those dysphagia regions of interest, as identified by a recent meta-analysis [31], describing both those areas for which there is a clear implication, identified by most studies, both those ones whose role is still under discussion. The hypothesized role in swallowing mechanisms is labelled as well.

Table 1. Cortical and subcortical regions involved in swallowing

Region of Interest (ROI) Selected For Analysis and Hypothesized Role in Swallowing		
ROI	Role	References
Primary Somatosensory, Motor and Motor Supplementary cortices (BA 1, 2, 3, 4, and 6)	Cortical processing of swallowing, including motor regulation and execution and sensorimotor control	Hamdy et al., Martin et al., Mosier and Bereznaya
Anterior cingulate (BA 24 and 32)	Higher order motor processing: swallowing movement planning and execution	Hamdy et al.
	Cognitive perceptual processes (attention and response selection)	Martin et al.
Orbitofrontal cortex (BA 10, 11, 12, 44, 45, 47)	Unclear	Mosier et al.
Parieto-Occipital cortex (BA 7,17, 18, 40)	Sensory processing of swallowing	Hadmi et al., Kern et al.
	Task-cue processing (not swallowing per se)	Toogood et al.
	Movement planning and execution	Mosier and Bereznaya

Table 1. (Continued)

Region of Interest (ROI) Selected For Analysis and Hypothesized Role in Swallowing		
ROI	Role	References
Insular Cortex	Processing of gustatory input	Daniels et al., Daniels and Foundas
	Intraoral sensory modulation	Mosier et al.
Internal Capsule	Functional connection of the cortical and brainstem nuclei via the corticobulbar tracts	Mosier et al.
Thalamus	Sensory and motor input processing via thalamocortical and thalamostriatal pathways	Daniel et al., Mosier et al.
Basal Ganglia (caudate and/or putamen)	Gating of Sensory Input	Mosier and Bereznaya, Daniel et al., Suzuki et al.
Cerebral Peduncle	Descending pathways from the cortex	Miller
Brainstem	Central pattern generator, swallowing regulation	Jean
Cerebellum	Regulation of adaptive coordination, sequencing, timing, learning and memory of motion	Zald and Pardo, Mosier and Bereznaya, Suzuki et al.

Initial studies in healthy volunteers described the midline swallowing muscles as bilaterally represented in the motor cortex, but in an asymmetric manner [12]. The Authors highlighted that in the majority of subjects, pharyngeal and esophageal projections from one hemisphere tended to be larger than those from the other one, suggesting a bilateral but asymmetric motor representation for swallowing musculature between the two hemispheres independently from handedness.

Using a complex paradigm, Hamdy et al. conducted a functional study of the cerebral loci that processes the human volitional swallowing with Positron Emission Tomography (PET) activation imaging, coupled in the same subjects with the single-pulse TMS with EMG recording in order to map the cortical motor representation of pharynx [32]. They firstly confirmed that the normal cortical representation of swallowing is bilateral and asymmetrical; moreover, the findings of sensorimotor cortex lateralization with PET were concordant with the cortical motor representation findings from TMS mapping, suggesting that also the motor aspects of swallowing show some degree of cortical asymmetry.

Lateralized cortical systems has also been indirectly investigate with the dual-task paradigm, by comparing the performances obtained at “baseline” with those obtained while performing specific tasks such as the finger-tapping, the line orientation and the repetition silent [33]. Swallowing was measured at baseline and during each of the three tasks to identify the different contribution of each hemisphere. Swallowing resulted to be impaired by concurrently performing the silent repetition, which mainly activates the left hemisphere, along with the line orientation, a visuospatial process that mostly activates the right hemisphere. Both the finger tapping of the right hand and that of the left hand, that selectively activate their contralateral sensorimotor cortex, interfere with swallowing. Moreover the

silent repetition leads to a significant change in the volume of the bolus, while the line orientation seems to have a negative impact on the number of swallows.

Even though it is now widely accepted the existence of a dominant hemisphere for swallowing in each individual, which is however independent from being left or right-handed, it has been not identified a hemisphere uniquely related to dysphagia so far; Notwithstanding, it has been suggested an association between the left hemisphere and the coordination of the oral voluntary phase, and between the right hemisphere and the pharyngeal automatic phase. In this light, evidences from literature suggest that there is an increased tendency for the pharynx to be involved, whether the damage is limited to the right hemisphere [34]; it is also intriguing that some Authors also described an asymmetrical cerebellar activation during swallowing, with a strong lateralization to the left cerebellar hemisphere and/or vermis; this seems to confirm the aforementioned hypothesis that the left brain is mostly involved in the coordination of the oral voluntary phase, while the right hemisphere seems to give a more relevant contribute to the pharyngeal automatic phase.

LESION LOCATION

From a historical point of view, for many years it was thought that a stroke must involve the brainstem or both hemispheres in order to cause dysphagia.

Since many studies over the last three decades have highlighted that also a single unilateral stroke is able to cause a swallowing impairment [34], many efforts have been made to identify those cortical and subcortical region whose damage could affect swallow mechanisms.

The importance of CPG in swallowing control is confirmed by the high incidence of dysphagia in patients with Wallenberg syndrome or lateral bulbar syndrome. Ischemia occurring at this level affects the nucleus of the solitary tract, the nucleus ambiguus and nuclei of the cranial nerves involved in swallowing. The impairment of the nucleus of the solitary tract can affect both the start and the synergy of the pharyngeal phase of swallowing; moreover, dysphagic patients with lesions at this level, have a hypoactivation of those brainstem areas resulting in a swallow impairment which mostly concerns the pharyngeal phase. Otherwise, the damage of the nucleus ambiguus leads to paralysis of the ipsilateral pharyngeal muscles, laryngeal and soft palate.

Flowers et al. [26] highlighted that other brainstem areas, such as the midbrain, the pons, and the lateral and medial portions of the bulb, are strongly correlated with post-stroke dysphagia. They reported as associated with dysphagia midbrain lesions in 6% of cases, pontine lesions in 45%, injuries in the medial bulb in 40%, and in the lateral bulb in 57% of cases; hence there would be a greater risk of swallowing dysfunction after pontine and bulbar stroke.

Also stroke involving insula seems to have a pivotal role in causing dysphagia, since lesions occurring at this level seem to be related to buccofacial apraxia and to a pharyngeal dysphagia strongly associated with aspiration [35].

Daniels and colleagues [27] also claimed that stroke lesions involving the insula may cause dysphagia by means of the disruption of the cortical-subcortical pathway which connect the anterior insula, both the primary and the supplementary motor cortex, the ventroposterior

medial nucleus of the thalamus and the nucleus of the solitary tract as well, all structures entrusted in the integrated mechanisms of oropharyngeal swallowing.

It was also observed that basal ganglia, and the associated neural pathways as well, play a role in the pathogenesis of post-stroke dysphagia by means of the progressive involvement of excitatory and inhibitory corticobulbar fibres. In this view, basal ganglia functionally connect the cerebral cortex and thalamus by providing a gate for sensory input entrusted in the motor control of the swallowing. Hence lesions of the basal ganglia and internal capsule are primarily associated with disorders of oral movement control, due to a premature leak of the bolus into the valleculae or the pyriform sinuses [12].

Since the last years, at a subcortical level, it has been a growing attention for the periventricular white matter [36]: the anterolateral portion of periventricular white matter contain both corticospinal descending motor fibers for the mouth and face, and ascending somatosensory ones as well. These ascending and descending fibers are embedded in an intrahemispheric cortico-cortical pathways: they cross the descending corticospinal fibers from the mouth/face representation within the ventrolateral precentral gyrus (motor cortex), and interconnect with specific cortical, subcortical, and brainstem regions involved in swallowing.

To date the role of cerebral small vessel disease (i.e., lacunar infarcts and white matter hyperintensities) in acute post-stroke dysphagia is still unclear. Notwithstanding it has been suggested that disruption of cortical-cortical and cortical-subcortical white matter connections may increase the risk of dysphagia and aspiration by lowering the threshold of input to the medullary swallowing center.

As far as it concerns cortical lesions, it is reported that a stroke that damages the contralateral precentral gyrus can produce deficits in motor control of the face, lips and tongue and compromise the pharyngeal peristalsis. This type of injury also cause deficits in attention and concentration, hence leading to alterations in the control of the swallowing.

Otherwise, despite several cortical areas have been described as related with swallowing impairment, a definite role in causing dysphagia has been not completely understood for all of them so far. Table 2 shows dysphagia regions of interest as identified by a recent meta-analysis [29]; Authors reported both those areas for which there is a clear implication, identified by most studies, both those ones whose role is still under discussion.

In conclusion, these evidences from the literature suggest that swallowing function is provided by a complex neural network bilaterally involving the perirolandic sensorimotor cortex which, together with other cortical structures, projects and receives afferences to the brainstem' Central Pattern Generator, through the basal ganglia, the thalamus and the periventricular white matter as well.

CPG provides in turn the basic motor plan for pharyngeal swallowing, and coordinative interactions between swallowing and respiration as well [22], by means of its compact clustering of bilateral cranial nerves nuclei and interneuronal network; through this network, sensory information entering the brainstem from the peripheral structures is integrated into a swallowing motor plan, and then transmitted to the motor nucleus for execution.

Finally, it is worthy of note that, despite the large number of studies, what cortical and subcortical areas are clearly related to dysphagia are not fully clarified so far, nor is it clear how and where their input are integrated and elaborated in order to create a voluntary and functional control of swallowing.

Table 2. Cortical regions involved in swallowing

Region of Interest (ROI) Selected for Analysis and Hypothesized Role in Swallowing		
ROI	Role	References
Primary Somatosensory, Primary and Supplementary Motor cortices (BA 1, 2, 3, 4, and 6)	Cortical processing of swallowing, including motor regulation and execution and sensorimotor control	Hamdy et al., Martin et al., Mosier and Bereznaya
Anterior cingulate (BA 24 and 32)	Higher order motor processing: swallowing movement planning and execution	Hamdy et al.
	Cognitive perceptual processes (attention and response selection)	Martin et al.
Orbitofrontal cortex (BA 10, 11, 12, 44, 45, 47)	Unclear	Mosier et al.
Parieto-Occipital cortex (BA 7,17, 18, 40)	Sensory processing of swallowing	Hadmi et al., Kern et al.
	Task-cue processing (not swallowing per se)	Toogood et al.
	Movement planning and execution	Mosier and Bereznaya
Temporopolar cortex (BA 22 e 38)	Unclear	Mosier et al.
Insular Cortex	Processing of gustatory input	Daniels et al., Daniels and Foundas
	Intraoral sensory modulation	Mosier et al.

DIAGNOSTIC ISSUES

As mentioned above, there is a great variability among reported post-stroke dysphagia incidence between studies, thereby resulting in a conflicting data concerning dysphagia risk factor, swallowing impairment recovery, and its clinical and neuroanatomical correlate as well.

This wide range of variability is mainly due to both diagnostic criteria and assessment methods issues.

With regard to the criteria used for post-stroke dysphagia diagnosis to be made, it is worth considering what exactly is meant by the term dysphagia: oropharyngeal dysphagia is probably best defined as a disruption of bolus flow through the mouth and pharynx, as resulting from multiple measures of bolus flow, structural movement and patient perception as well [37].

Several studies aimed to investigate post-stroke dysphagia actually referred to aspiration, a related but distinct term which means the penetration of food material into the airways and below the true vocal cords.

From this point of view this seems somehow misleading, firstly because it is well known that dysphagia may occurs without aspiration; moreover, even with an instrumental evaluation, the incidence of dysphagia may be overestimated if its identification is based on

laryngeal penetration or single occurrences of aspiration [28], as also healthy subjects may exhibit laryngeal penetration and sometimes aspiration as well [20].

Finally, by focusing on the overt sign of aspiration to diagnose post-stroke dysphagia, such as cough or voice change, the aforementioned silent aspiration would be missed, with a relevant increase in relative risk of pneumonia and a poorer clinical outcomes.

Concerning the implied diagnostic methods, the post-stroke dysphagia incidence is reported to be lower with the adoption of less accurate and precise screening tests (37%-45%), higher when clinical tests are used (water swallow test, 51%-55%) and even higher when dysphagia diagnosis is based on instrumental tests (fiberoptic endoscopic evaluation of swallowing or videofluoroscopy, 64%-78%) [3].

Among the instrumental assessment tools, videofluoroscopic evaluation (VSE) and fiberoptic endoscopic evaluation of swallowing (FEES) are thought to be the current gold-standard. These techniques are equally sensitive to the detection of post-stroke aspiration and pneumonia and likewise useful in the evaluation of oropharyngeal dysphagia [38].

However, these tests have several practical limits. VFS is a relatively complex task both to perform and to interpret; test-test variability has been found within normal subjects undergoing this exam and interjudge agreement between different radiologists for VFS assessment can be very variable, probably reflecting their different skill or experience [39, 40]. Moreover for VFS to be undertaken, patients have to cooperate or at least be able to sit upright, which does not replicate physiological conditions. Further limitations are imposed by radiation exposure involved which, although regarded as acceptable, makes it an inappropriate test to repeat frequently to monitor changes [38].

Otherwise, FEES is a safe and well tolerated assessment conducted at bedside thereby allowing timely scheduling for both the endoscopist and patient [41, 42]; it is however an operator-dependent examination requiring both operator and specialized equipment expertise; besides it provides limited information about the oral and esophageal stages since aspiration cannot be seen directly, being inferred from residue left after swallowing or ejected after coughing [43].

Given the aforementioned limitations of those instrumental tools and considering that both VFS and FEES are not available in many clinical settings in which stroke patients are managed, there is a need of a valid, reliable, and feasible screening tool for the early assessment of dysphagia.

In this point of view, bedside clinical assessment of swallowing (BSA) has become a widely used initial test to identify those patients who would benefit from a more detailed assessment of their swallowing, such as VFSS or FEES [44].

By definition, this should be simple, quick to perform at bedside and sensitive enough to accurately identify stroke patients with risk of swallowing impairment. As a matter of fact a high sensitivity is required because of the increased morbidity and mortality associated with aspiration, which necessitates requirements for low false-negative results [1].

Nonetheless, the Joint Commission recently excluded swallowing screening as a performance measure for Primary Stroke Center certification [45]; moreover, although there are currently many bedside tests, clinical bedside assessment has been shown to miss up to 40% of those patients who aspirate; this is mostly because of the lack of systematically defined standards for what constitutes a valid swallowing screening tool, being still unclear which of these have the best psychometric and feasible properties and are easy to administer.

The following are the features most widely used in the literature as a part of the bedside evaluation of dysphagia.

Water Swallow Test (WST)

This test used water administered in various aliquots and ways as a trial swallow, while monitoring coughing and choking during and after swallowing. Indeed in the literature, the number of trials varied, together with water volumes administered which ranged from 5 to 150 mL. Different features has been also monitorized during WST, depending on the different study designs; Leder and colleagues found that the presence of 2 of 6 features (dysphonia, dysarthria, abnormal volitional cough, cough after swallow, abnormal gag reflex, and voice change after swallow) predicted greater dysphagia severity on VF [42]. When analyzing studies that used WST to evaluate aspiration and/or penetration [39, 44, 46-48], sensitivity ranged from 27% to 85%, while specificity ranged from 50% to 88% [49]. Several Authors [50-52] also used different viscosities textures (i.e., liquids, semi-solids and solids) while performing WST; although they reached an higher sensitivity and specificity, the assessment feasibility became lower because of the need of both considerable quantities of text materials and the time required for the test to be done.

Bulbar Function Assessment

WST effectiveness can otherwise be increased by careful assessment of bulbar function (i.e., abnormalities of pharyngeal sensation and reduced cough reflex, along with monitoring laryngeal elevation during swallowing. This is mostly because cough and wet voice in response to a WST, together with abnormal palatal sensation, are thought to be essential predictors of prolonged dysphagia [53], aspiration [1] and particularly useful in detecting silent aspiration [54].

Smithard and colleagues also identified weak voluntary cough after drinking different volumes of water, along with impaired consciousness level, as independent predictor for aspiration [39].

Moreover in acute stroke patients with dysphagia, a sensory deficit in the pharynx and supraglottic has been demonstrated by now. This sensory deficit is thought to play a pivotal role in the development of aspiration both directly, by reduced stimulus detection and discriminative capacity in the laryngopharynx, and indirectly, in impaired triggering of motor actions [53].

In this light many Authors searched for a reliable clinical or instrumental tool, which could allow a valid bulbar function assessment. Smith-Hammond and colleagues found that in stroke patients, objective measures of voluntary cough (i.e., duration, volume and peak flow of the inspiration phase, compression phase duration, peak flow and rise time for the expulsive phase, and volume acceleration) are strongly related to aspiration [55]; in this study it was also stated that subjective assessments of voluntary cough had otherwise poor performance.

Sensory capacity of the laryngopharynx has been also determined by endoscopically delivering discrete air pulse stimuli directed at mucosa innervated by the internal branch of

the superior laryngeal nerve. Given that air pulses test has been proven to detect laryngo-pharyngeal sensory deficits also in stroke patients with no clinical dysphagia [41], this examination might be essential for the detection of the so-called silent aspirators. Notwithstanding, it also appears to be not such a practical and reliable tool mostly because of it requires both an endoscopically delivered stimulus and an air pulse instrument.

Pulse Oxymetry and Silent Aspiration

Although pulse oxymetry has been criticized in the literature as a valid screening tool [56, 57], many studies focused on oxygen desaturation to evaluate aspiration/penetration since it has been shown to detect silent aspiration [44, 46, 52]. Depending on the decrease between the baseline oxygen saturation and the minimum saturation during the 120 sec, a pulse oxymetry decrease of greater than or equal to 2% has been widely considered as the endpoint. The sensitivity of this test so ranges from 56% to 87% while specificity from 39% to 97%, both depending on the different studies [39, 48]. Moreover, two different studies tested a combination of a swallow and oxygen desaturation test, by using coughing, choking, voice change or $\geq 2\%$ desaturation as the endpoints [44, 46].

With this paradigm, they reached a sensitivity of 94% and 98%, and a specificity of 63% and 70%, respectively [58]. In agreement with these data, in a recent review performed by Bours and colleagues it is stated that, by considering both the best psychometric properties and the feasibility of the screening methods, WST combined with pulse oxymetry (considering choking, coughing, voice change and desaturation $\geq 2\%$ as fixed endpoints), should be considered, to date, the most promising results as a screening tool in terms of validity, reliability and feasibility [49].

Nevertheless, although the most important international guidelines stress the importance of Screening of swallowing before the administration of food, liquid, or medication in patients with stroke, they do not recommend a specific screening method so far [59].

There is not even consensus in the literature, regarding either the best type of swallowing assessment to use, i.e., clinical or instrumental, or what specific characteristics acute stroke patients should have in order to benefit from either type of testing [42].

Hence WST appears as a simple tool, quick to perform at bedside and sensitive enough to identify stroke patients with risk of swallowing impairment.

CLINICAL AND NEUROANATOMICAL PREDICTORS OF POST-STROKE DYSPHAGIA

A recent study was conducted in order to assess clinical and neuroanatomical predictors of dysphagia following stroke [60]: the main purpose of the study was to investigate a sample of consecutive patients with ischemic and hemorrhagic stroke to evaluate incidence, prognosis, clinical and neuroradiological correlates of post-stroke dysphagia. Consecutive patients with acute ischemic or hemorrhagic stroke were consecutive recruited and followed-up after 14 days, in order to assess post-stroke swallowing impairment; according to the swallowing function, patients were then classified as: a) persistent dysphagic b) non-

persistent dysphagic or c) non-dysphagic. The role of leukoaraiosis in the pathogenesis, persistence and prognosis of post-stroke dysphagia is also discussed.

Patients who presented dysphagia were significantly older than those without; thus, elderly patients with stroke may have more swallowing alterations due to the reduced cough reflex and alteration of swallowing/ breathing coordination [61].

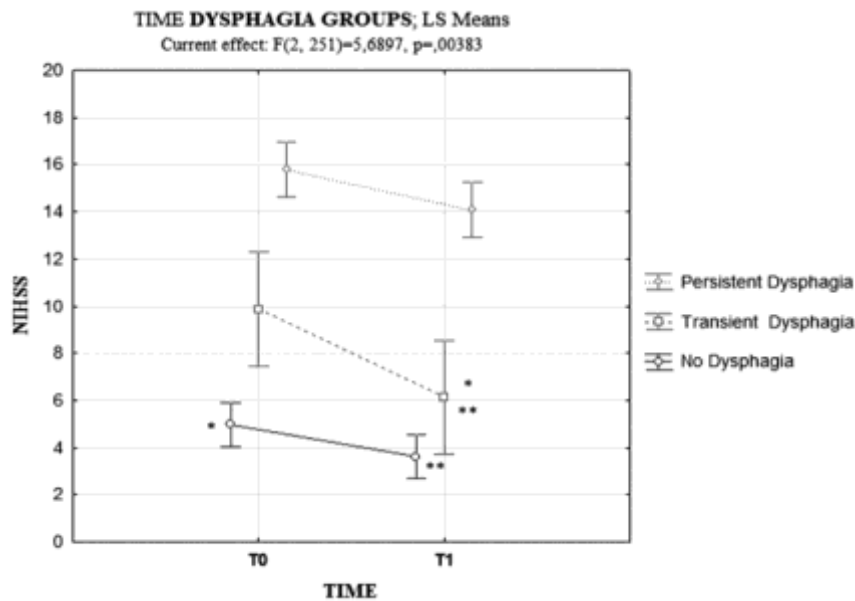
Dysphagia was influenced by the lesion size, but no differences were identified in the involvement of right or left hemisphere. In this view, the majority of studies performed on stroke patients did not observe any relation between the presence of dysphagia and side of hemispheric location [62-64]. However, it is known that lesions located in the right hemisphere may cause more pharyngeal changes and that lesions in the left hemisphere cause more changes in the oral phase of swallowing [65].

The NIHSS score at admission was higher in dysphagic patients. With this regard, the three different pattern of dysphagia (i.e., absent, transient or persistent) were compared over time regarding the stroke severity at admission, data from the ANOVARM after Tukey HSD post hoc analysis showed (Figure 1):

1. Patients with persistent dysphagia (PD) vs. patients with transient dysphagia (TD):
 - PD showed a significantly higher T0 and T1 NIHSS score with respect to both T0 ($p < 0.001$, $p = 0.023$ respectively) and to T1 ($p < 0.001$, $p < 0.001$ respectively) NIHSS scores of TD.
1. Patients with persistent dysphagia (PD) vs. no dysphagic patients (ND):
 - PD showed a significantly higher T0 and T1 NIHSS score with respect to both T0 ($p < 0.001$, $p < 0.001$ respectively) and to T1 ($p < 0.001$, $p < 0.001$ respectively) NIHSS scores of ND.
2. Patients with transient dysphagia (TD) vs. no dysphagic patients (ND):
 - TD showed a significantly higher T0 NIHSS score with respect to both T0 ($p = 0.002$) and to T1 ($p < 0.001$) NIHSS scores of ND, whereas T1 NIHSS of TD did not differ from both T0 ($p = 0.95$) and to T1 ($p = 0.38$) NIHSS scores of ND.

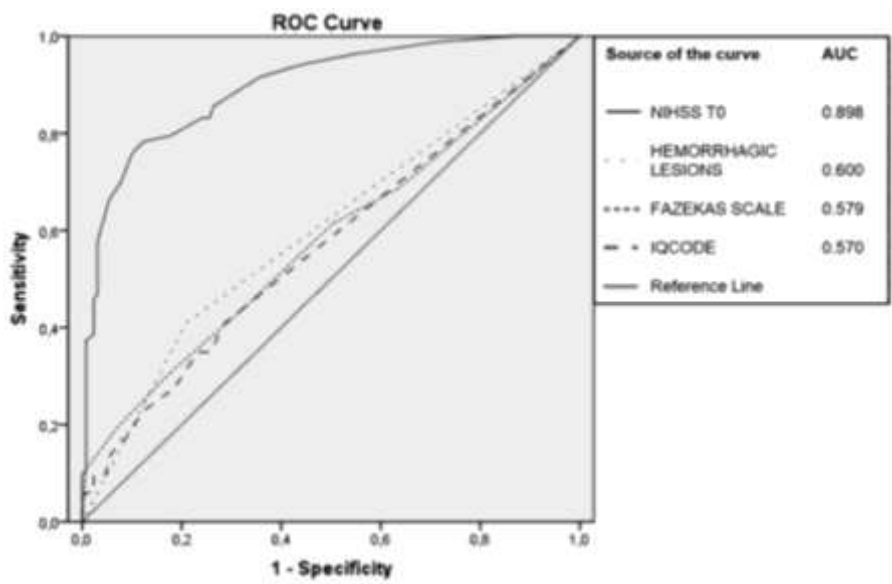
In other words, stroke severity was significantly higher in patients with persistent dysphagia with respect to those with transient dysphagia or without dysphagia, both at admission and at T1. Moreover, those patients with a transient pattern of dysphagia showed a sensibly improved NIHSS score at T1, which became similar, on average, to that of patients without swallowing impairment upon admission.

In the multivariable logistic analysis, stroke severity at admission was found to be an independent predictor of a persistent pattern of post-stroke dysphagia. The multivariable logistic model also allowed to represent ROC (receiver operating characteristics) curves and evaluate the predictivity of each significant variable using the AUC (Area Under the Curve) value. In this view, looking at ROC analysis, the T0 NIHSS score of 11.5 was found to be the best predictive value of persistent dysphagia, with a specificity of 90.1% and a sensitivity of 72.4%; the reported AUC was 0.898, which means that stroke severity at admission allowed to correctly classifying about 90 of 100 dysphagic patients in a persistent or non-persistent pattern. Hence, a $\text{NIHSS} \geq 12$ would be suggested as cut-off value in order to predict, upon admission, those patients who will probably remain dysphagic after 14 days follow-up. Figure 2 shows the ROC curves and the respective AUC.



Tukey post hoc analysis showed a significant difference between all groups (longitudinally compared among NIHSS) but: *p = 0.95, **p = 0.38. See the text for the details.

Figure 1. ANOVA for Repeated Measures.



AUC = Area Under the Curve.

Figure 2. ROC Analysis: persistent dysphagia.

Dysphagia was also more frequent in patients with hemorrhagic stroke; there are little available data in the literature reporting a different risk factor for dysphagia depending on stroke type. Paciaroni et al. [63] found, in a univariate comparison, an increased frequency of dysphagia in hemorrhagic stroke patients compared to ischemic ones. They suppose that it

could be related to stroke severity. Our results confirm this datum, since the Spearman's Rank Correlation revealed a strong statistical dependence between stroke severity at admission and hemorrhagic stroke. Looking at logistic regression analysis, the stroke type also appears an independent predictor for post-stroke dysphagia (OR 2.5); nonetheless hemorrhagic patients present a more than three-folded risk of persistent dysphagia. Hence, further research is needed in order to investigate a possible intrinsic mechanism linking dysphagia and hemorrhagic stroke.

Patients with high IQCODE score, which means a pre-stroke cognitive impairment, had more chances to present swallowing dysfunction; moreover, logistic regression analysis showed that the IQCODE score is an independent risk factor for post-stroke dysphagia and persistent dysphagia as well. These results are in agreement with previous reports which stated that brain lesions that cause decline in cognitive function, such as concentration and attention, may impair the control of swallowing [66].

Dysphagia was more frequent in patients with insular stroke, as well as in those with stroke involving frontal and temporal lobes, and basal ganglia. As a matter of fact, all the aforementioned structures are involved in the neural control of swallowing; in particular, insula is known to be closely connected both with cortical and subcortical pathways involved in the neural control of swallowing. Nevertheless, none of those structures was found to be an independent risk factor for dysphagia. In the case of insular stroke, this seems mostly due to the small number of pure insular stroke.

Otherwise, concerning the others aforementioned structures who failed to reach statistical significance once logistic regression analysis has been performed, this datum could suggest that cortical-subcortical pathways involved in the swallowing function are probably more important in causing dysphagia after stroke than a single structure "*per se*."

This hypothesis could be corroborated by analyzing the contributions of white matter to swallowing behavior. Interestingly, the Authors observed an independent correlation between post stroke dysphagia and leukoaraiosis severity as well as a strong association between leukoaraiosis and previous cognitive impairment. Recent studies reported that subcortical white matter connections play an important role in the pathogenesis of dysphagia: disruption of cortical-cortical and cortical-subcortical white matter connections seems to increase the risk of dysphagia and aspiration pneumonia by lowering the threshold of input to the medullary swallowing center.

These data, as well as those showing the role of a decline in cognitive function, suggest that swallowing impairment after stroke might be not just a simple pure motor disturbance, but also a more complex phenomenon. In this context, one might speculate that dysphagia may result from the combined effects of an acute focal damage and a preexisting impairment of the subcortical white matter connecting pathways. In other words, we might think of dysphagia as a kind of deficit in executive functions, which we know to be particularly associated with leukoaraiosis and cognitive disorders [67], and of course age [61].

Finally, pneumonia is among the most common medical complication after stroke, affecting up to one third of patients and accounting for nearly 35% of post-stroke deaths [2, 68]. Data from the aforementioned study [60] also suggest that stroke and pneumonia are associated with two-fold: on the one hand, pneumonia appears to be strongly linked to the presence of dysphagia at admission. In fact, among those patients who developed pneumonia during the entire hospitalization, 38 (93%) were dysphagic at admission. On the other hand, the persistence of dysphagia after 14 days was closely linked with the occurrence of

pneumonia ($p < 0.001$), with 36 patients (88%) with pneumonia who showed a persistent pattern of dysphagia.

RECOVERY FROM POST-STROKE DYSPHAGIA AND PERSISTENT DYSPHAGIA

The nervous system affected by a stroke damage is thought to reserve a variable ability to somehow reorganize damaged pathways and structures, allowing an effective recovery from swallowing impairment.

In this view, recent findings seem to suggest that the reorganization of cortical maps actually represents a central features in recovery from dysphagia after stroke.

In a MEG study Teismann and colleagues [69] analysed the swallowing-related activation areas both in stroke patients with dysphagia, in those patients without dysphagia and in healthy control as well. In the acute phase of stroke, it was described:

Patients with hemispheric stroke: while those patients without dysphagia reported an activation of the bilateral hemispheres, patients with post-stroke dysphagia are described to have a decreased cortical activation of the ipsilesional sensorimotor areas, and a near extinguished activation of the contralesional hemisphere; the whole sample of hemispheric stroke patients also showed an activation of the prefrontal cortex (BA 9) which was neither seen in healthy subjects nor in patients with brainstem stroke.

Patients with brainstem stroke: in both patients with and without dysphagia it was observed a reduced cortical activation, due to cortical compensation of a subcortical caused dysphagia, as well as a strong right hemispheric lateralization; the Authors speculated that this phenomenon may be related to “diaschisis.” This concept was developed by Von Monakow in 1914 and refers to the fact that the acute lesion may cause a damage of other areas functionally linked.

In healthy controls they observed a bilateral activation, mostly involving both the primary and supplementary motor cortex (BA 4 and 6). Other activation areas was represented by the primary and secondary sensory areas (BA 1, 2, 3, 4, 5, 6 and 7).

Hence, in conclusion, a strongly reduced cortical activation was bilaterally observed in stroke patients with dysphagia, probably due to an initial cortical shock after stroke.

What is absolutely remarkable, is that the activation of contralesional areas changed over time: in contrast to a strongly reduced activation in the acute phase, in the subacute phase of stroke (mean 12 days after stroke) this pattern changed to an increased brain activation of the contralesional hemisphere [69].

This datum seems to be crucial in order to understand the complex mechanisms leading to recovery from swallowing impairment after stroke.

In this point of view, it has been postulated that the recovery mechanisms from post-stroke dysphagia involve changes in neural networks already different from those described in the motor recovery after stroke.

After motor stroke, a reduced cortico-cortical connectivity in the affected hemisphere has been already described, as well as disinhibition phenomena (i.e., increased task-related BOLD activity) in the unaffected (i.e., contralesional) hemisphere [70]. Notwithstanding, the role of the contralesional primary motor cortex (M1) for motor recovery remain somehow

controversial: it is reported that enhanced activity in contralesional M1 might even exert a negative influence on the motor recovery of the paretic hand, probably due to transcallosal inhibition mechanisms between the primary motor cortex of both hemispheres. This “inter-hemispheric inhibition” is otherwise confirmed by several TMS-fMRI studies which show that stroke patients with motor impairment may benefit from contralesional M1 inhibition [71]. Hence, it is suggested that a strong engagement of the unaffected hemisphere may represent an indicator of inefficient cortical reorganization, thereby exerting a detrimental effect on motor recovery after stroke.

On the contrary, recent data shows that recovery from post-stroke dysphagia follows a reorganization of the unaffected motor cortex.

Hamdy and colleagues [72], by coupling fMRI analysis with TMS-evoked EMG responses, described a different compensatory reorganization between hemispheres during the recovery of swallowing and limb function: while improvements in limb function were associated with an increase in cortical representation in the damaged hemisphere, patients who recovered from dysphagia, showed an increased pharyngeal representation in their cortical maps over the unaffected hemisphere, both at one and at three month after stroke, without change in the affected hemisphere [37,62]. In contrast, those patients who remained dysphagic, did not show any variation in their pharyngeal map.

The most interesting findings is that this cortical change seems to appear before any improvement in swallowing function, suggesting that the change in cortical excitability for pharynx is not merely the consequence of the normal swallow activity resumption, but it actually represents the drive for dysphagia recovery.

Moreover, they also found that pharyngeal responses from the unaffected hemisphere showed a marked increase in both the amplitude and area of representation over time, without concurrent shift in latency; this datum suggest an alteration in the excitability of cortex rather than more caudal brain regions, such as brainstem central pattern generator or bulbar motoneurons.

It is intriguing that it was also described a different compensatory reorganization between hemispheres during the recovery of swallowing and limb function.

It could be then speculated that this finding supports the hypothesis of a bilateral and asymmetric representation of swallowing: since swallowing musculature, unlike limb musculature, is represented on both hemispheres, although a damage to the dominant hemisphere for swallowing may cause dysphagia, there remains within the intact hemisphere the capacity for reorganization, by exerting a sufficient control over brainstem centers to drive swallowing recovery.

Notwithstanding, about half of stroke patients remain dysphagic after seven days from symptoms onset, and approximately 13-15% [73] of patients has persistent dysphagia after six months.

To date, it is unclear why some stroke patients develop a persistent swallowing impairment after stroke or, in other words, if the failure in recovery from dysphagia is due to a stroke involving the dominant swallowing hemisphere, rather than a swallowing key region, or even to a less efficient contralesional hemisphere activation, perhaps due to an insufficient bilateralism in cortical representation of swallowing.

In this view, many efforts have been made in order to identify those patients who are at high risk for developing a persistent dysphagia after stroke, and therefore to identify those patients who may benefit from a more detailed assessment of their swallowing function.

Nevertheless, clinical and neuroradiological correlates of post-stroke dysphagia remain still unclear, and even fewer data are available on predictors of persistent dysphagia so far.

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Main Publications Last 3 Years:

Chapter 91

OXYTOCIN AS A TREATMENT OPTION IN RIGHT HEMISPHERE STROKE

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ABSTRACT

Impairment of empathy or social-emotional functioning are a disabling central feature in right hemisphere stroke, for which there is currently no proven effective treatment. Oxytocin may be a viable treatment to ameliorate this deficit. A decade of oxytocin research in humans has shown us that the hormone may not have prosocial effects in all situations across all people. Care must be taken to ensure that factors that influence its effects are addressed in a treatment paradigm. Long term oxytocin treatment paired with social cognitive training in a controlled clinical setting may prove to be an effective implementation of the peptide. Future work may have to consider problems in current statistical analysis, oxytocin delivery, and understanding of gender effects.

INTRODUCTION

The importance of empathy in our lives is apparent. It plays a role in nearly all human social interactions and is critical in the maintenance of close human relationships. Impairments in empathy have been well-documented in several neurological and neuropsychiatric diseases such as autism, schizophrenia, frontotemporal dementia, and traumatic brain injury (Baron-Cohen et al., 1985; Sacks et al., 2013; Hillis, 2014). With some

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of the earliest reports appearing in the late 1970s into the 1980s, patients with right hemisphere stroke have also been reported to have a range of pragmatic social deficits from difficulties with prosodic recognition and production to impairments in theory of mind and perspective taking (Hier et al., 1983). These deficits can have negative consequences such as social isolation or decreased marital satisfaction that may impact quality of life (Blonder et al., 2012). In a 2014 study from our Stroke Prevention and Recovery Center, residual impairment empathy was found to be one of the top concerns for 50% of spouses or adult children of right hemisphere stroke survivors (Hillis and Tippett, 2014). This impairment may also prevent patients from accessing familial social support, which worsen long term outcomes of patients (Grant et al., 2006). Despite the apparent impact and importance of empathic deficits, there are currently limited proven treatments available for right hemisphere stroke patients.

In considering a potential treatment for social-emotional deficits, the goal of such a treatment must be clearly defined. From our perspective, a treatment for these deficits should ideally return patients to a state where they can make emotional and prosocial decisions the same way that they were made before the stroke. In essence, an ideal treatment should fully repair the damage done by a stroke to the social functioning of a patient and return that patient to a pre-stroke functioning level. To this end, physiological and psychological experience of emotions must be fully restored along with social-cognitive functions where deficits exist. Actions beyond this are not within the scope of the neurological perspective presented here and fall under the purview of psychology and psychiatry.

Our aim in this chapter is to introduce oxytocin as a potential treatment option to accomplish this goal of the restoration of social cognitive functioning after stroke. We will begin with some background on stroke and a discussion of empathy. Afterwards, we will transition to a discussion of oxytocin research in humans, its potential benefits for stroke patients, and practical considerations that are needed to plan a future oxytocin clinical trial.

OVERVIEW OF RIGHT HEMISPHERE STROKE

A stroke is defined as a sudden focal neurological deficit due to a disruption in the blood supply to a specific area of the brain. Approximately 795,000 people each year experience a stroke in the United States, and stroke is a leading cause of long-term disability (Mozaffarian et al., 2015). Disruption of blood flow in stroke caused by vessel blockage, known as ischemic stroke, accounts for 87% of cases (Grysiewicz et al., 2008). Stroke is also caused by blood vessel rupture, known as hemorrhagic stroke, which accounts for the remaining cases.

Due to the lateralization and functional organization of the brain, lesions caused by stroke can cause dramatically different deficits depending on the brain structures and hemispheres affected by the stroke. For instance, speech and language issues are most common in patients with left hemisphere stroke and are not as prevalent in patients with right hemisphere stroke, with the precise deficits being further dependent on the exact left hemisphere regions affected (for review see Hillis, 2007). In contrast, deficits in visual and spatial processing are very common in patients with right hemisphere but not as prevalent (or at least not as severe) in left hemisphere stroke patients (Hier et al., 1983). The focus of this chapter will be on empathic deficits common in patients with right hemisphere stroke (Happé et al., 1999).

Despite these differences, left and right hemisphere stroke patients often experience some more ubiquitous symptoms in the form of contralateral paralysis and some generalized cognitive deficits, though the extent of these symptoms are highly variable between patients (Mozaffarian et al., 2015).

The deficits incurred as a result of stroke are by no means static. Considerable plasticity leads to spontaneous recovery across cognitive, sensory, and motor domains (Langhorne et al., 2009; Sebastian et al., 2016; Murphy and Corbett 2009). A significant amount of the current research on stroke recovery focuses on understanding, predicting, and enhancing the brain's natural ability to repair damage. Treatment approaches that may stimulate this activity such as transcranial direct current stimulation (tDCS), medications, and behavioral therapies (often in combination) are currently being tested as a way to enhance recovery (Shlaug et al., 2008; Baker et al., 2010).

DEFINING EMPATHY

Given the highly subjective nature of empathy, it is important to first understand how empathy is characterized by psychologists and cognitive neuroscientists. Much of the non-human primate and developmental literature has split empathy into two separate components: an affective component and a cognitive component (Shamay-Tsoory, 2010).

The affective component, an evolutionarily conserved feature in much of mammalian lineage, encompasses simple emotion recognition skills and a phenomena known as emotional contagion (Ze et al., 2014). Emotional contagion describes a process where an individual's emotional state will shift towards the perceived affective state of another person (Melloni et al., 2014). It is believed that emotional contagion can occur without an explicit cognitive attribution of the acquired affective state to another person or an explicit understanding of why the other person is displaying a specific affective state. For instance, a young child may cry if someone around them is clearly upset, but they may not be aware of the reasons underlying the other person's feelings and they may not attribute the source of their own sadness to the other person.

In contrast, the cognitive component of empathy has been conceptualized as a uniquely human or uniquely primate element of empathy that involves more intricate cognitive understandings of another's emotional state (Shamay-Tsoory, 2010). It is contingent on an ability known as theory of mind, which is the ability to understand the mental state of another as separate from one's own emotional state with independent goals, intentions, and feelings. Cognitive components of empathy encompass knowledge of the cause of another person's emotional state and an understanding of the implications of that state (Hillis, 2014). If emotional contagion is an "early" form of empathic ability, the cognitive aspects are more akin to the complex social-emotional reasoning that we see develop as a person develops from child to adult.

However, it is our view that neither the affective nor cognitive components of empathy operate alone during real-world social interaction in adults. This is because nuanced human empathic reactions to social situations involving other people are predicated on a complex interaction between these two components (Ochsner and Gross, 2005). For example, the response evoked during emotional contagion can be modified based on cognitive insight and

perception, and an affective state evoked via contagion can similarly modify our cognitive interpretations. Given this simultaneous feedback between cognition and contagion, we combine affective and cognitive empathic skills into a single but multicomponent construct, which we term emotional empathy. Emotional empathy is then a unitary experience of empathy that is the sum of the interaction of affective and cognitive components of empathy and the underlying processes of those two constructs.

Considering this broader and more encompassing view of emotional empathy as a single entity rather than as two separate constructs is key in the context of discussing prosocial behavior and social functioning in right hemisphere stroke patients. Though the link between empathy and prosociality is beyond the scope of this chapter, it is reasonable to assume that a disruption in either contagion or perspective taking would cause some information or motivation to be lost especially because of the interaction between the two (Bzdok et al., 2012). Thus, a return to premorbid social functioning is contingent on ensuring recovery of the full spectrum of emotional empathy. While the exact nature of premorbid social functioning will be different for each patient, a reorganized and effective neural system for emotional empathy will be necessary to ensure that similar social decisions can be made as they were before.

It is important to note that it is possible for right hemisphere stroke patients to experience social functioning deficits that are not due to damage to emotional empathy *per se*. The most common example in this patient population is aprosodia. Prosody refers to elements of spoken language such as timing, loudness, stress, or frequency modulation that affect dramatically the meaning of what is said (Ross and Monnot, 2008). Right hemisphere stroke generally disrupts expression or recognition of affective prosody—prosody that communicates the feelings of the speaker or their attitude (Blonder et al., 2005). Deficits in recognition and production of affective prosody have been shown to cause communicative difficulties that and can strain personal relationships (Blonder et al., 2012). As one might imagine, not being able to easily extract affective prosodic information could result in less information reaching the emotional empathic process though the impaired social interaction is not due to an empathic deficit *per se*. In addition, if a patient is unable to convey prosodic affective information in speech they may sound apathetic even if they are not. A parallel process may occur with impaired recognition or display of facial expressions, where a patient would face difficulties with empathic functioning that do not arise from a strictly empathic deficit. Thus, loss of function in either emotional information input or emotional expression output can pose major issues for the social functioning of patients. Given this conception of empathy and empathic deficits, let us turn our attention to a discussion of the modern understanding of oxytocin. If we aim to use oxytocin as a treatment, we must first understand how it functions.

TOWARDS A NUANCED UNDERSTANDING OF OXYTOCIN

Oxytocin is a peptide hormone released by the posterior pituitary that has historically been commonly associated with its biological role in mammalian childbirth and lactation. However, early work also showed that the hormone also appears to influence mammalian animal behaviors such as maternal behavior and pair-bonding (Pedersen & Prange, 1979;

Pedersen et al., 1982; Williams et al., 1994). These early studies in rats and prairie voles were able to establish the link between oxytocin and behavior because they could directly inject oxytocin into central nervous system structures and observe behavioral changes. Similar results have been replicated in other mammals such as sheep and non-human primates (Costa et al., 1996; Smith et al., 2009). Given these strong results, interest in oxytocin's potentially prosocial effects in humans has rapidly grown over the past two decades. Unfortunately, many of the methodologies used in animal work involved invasive surgeries that were not appropriate for human use. This challenge was overcome when it was found that oxytocin inhaled intranasally could increase central nervous system levels of the peptide hormone (Born et al., 2002).

Since the discovery of the effects of intranasal oxytocin, the number of oxytocin studies in humans has increased considerably. One of the first major studies came in 2005 from Kosfeld et al. with the discovery, in a double-blind placebo-controlled study, that intranasally administered oxytocin may facilitate trust in humans. Their experiment, known as a trust game, involves an "investor" participant and a "trustee" participant each starting with a certain amount of money. The investor can then choose to transfer some of their capital to the trustee. Any money transferred to the trustee triples in value, meaning that the trustee would receive 24 monetary units if they were given eight. At this point, the trustee can choose how much to transfer back to the investor, if anything. The game poses a dilemma for the investor because their trust in the other player could result in ending the game with less money than they started the game with. Importantly, Kosfeld et al. found that investors who were administered oxytocin were more likely to offer more money to trustees than investors given placebo. The authors argued that these data showed increased trust behavior on the part of the investors. This paper had a major impact on the field because it showed that administering oxytocin could produce prosocial effects.

Several other studies published around the same time found similarly prosocial effects. For instance, Domes et al. (2007) found that oxytocin was able to increase the ability to infer internal states based on pictures of the eye region of faces in the Reading the Mind in the Eyes Test (RMET), a key empathic skill set. Early fMRI work suggested that oxytocin's effects may be due to down-regulation of the amygdala, decreasing activation to produce an analgesic effect that promotes social approach behavior (Kirsch et al., 2005; Domes et al., 2007b). A study showing the early promise of oxytocin as a treatment option in neuropsychiatric disorders found that oxytocin facilitated processing and retention of social information in patients with autism (Hollander et al., 2007).

Taken together, these results painted a very optimistic picture of oxytocin that would drive research and popular perception into our current decade. The findings of many of these early oxytocin trials suggested a very broad range of prosocial effects hence perceptions of oxytocin as a "moral molecule" or "prosocial peptide". In recent years, the field has since shifted away from this perception with many questioning this overly simplistic characterization of oxytocin as a kind of "social panacea" (Bartz, 2016). One of the major concerns raised by the current literature revolves around the fact that large scale meta-analyses show a great deal of inconsistency in results across studies. Null results or even antisocial results are obtained in oxytocin trials too frequently in the literature to be explained solely by random error (Bartz et al., 2011b; Bartz, 2016). In addition to these concerns, other authors raise issue with statistical and methodological aspects of individual oxytocin studies (Walum et al., 2016; Lend and Ludwig, 2015).

While the plurality of oxytocin's observed effects is not yet fully understood, there is some consensus on a three pronged model presented by Bartz, Zaki, Bolger, and Oschner in their seminal 2011 review. Bartz et al. present the wide spectrum of results from null to prosocial to antisocial as being explainable under three different mechanisms. The first proposed mechanism is that oxytocin may act as an anxiolytic, reducing social anxiety and increasing prosociality through this action. It is important to note that this cannot easily explain the antisocial effects of oxytocin and increases in performance on some social cognitive measures. The second mechanistic proposal is that oxytocin activates a desire to affiliate with others. This mechanism would explain increased social cognitive performance and leaves room for context dependent and person-dependent effects. The third proposed mechanism is that oxytocin increases the salience of social stimuli. This would essentially enhance cognitive access to social-emotional information; the context under which the information is received and individual social strategies would likely modulate the response.

Though many critics of the circa 2007 view of oxytocin cite the contemporary view as a major departure from the "prosocial peptide" era the complexity of the effects of oxytocin were alluded to in the early human literature. For instance, in the Kosfeld trust study, the investigators reported that oxytocin increased the trust of the investors, but oxytocin administration did not increase the generosity of the trustees when deciding how much to transfer back to the investors. Here, it is clear that oxytocin was not a blanket prosocial drug that increased all positive social behavior. For the Domes et al. study, though it is often referred as a study that showed universal increased emotional inference ability on the RMET upon oxytocin administration, the effect of oxytocin was only significant for items considered to be very difficult. The overall significant increase in performance was driven by the more challenging items with no significant difference in performance on simpler items. For this reason, these results imply that administering oxytocin may not actually impact simple and routine empathic judgments that a person frequently encounters, demonstrating a narrower effectiveness for the peptide. Ultimately, these original studies should be taken as a proof of concept that oxytocin can have prosocial effects. Further work on oxytocin has revealed important context dependence and person-dependent nature of its effects.

A paper published in 2010 by Declerck, Boone, and Kiyonari is perhaps one of the most well-known examples of the context dependence effect. In this study, participants played two monetary games, one that encouraged cooperative action and one that was structured as a prisoner's dilemma. In the cooperative action game, theoretically the best possible outcome for either player would be if both cooperated, and choosing a selfish action would result in a less favorable outcome for both players. In the prisoner's dilemma, choosing the selfish action results in the best outcome leaving little incentive to cooperate. A key aspect of the study is that half the participants were allowed to meet their partners for the game beforehand, while the other half were not. As expected, participants were overall less likely to cooperate in the prisoner's dilemma scenario compared to the cooperation scenario. However, oxytocin actually decreased the willingness to cooperate for players who had not met their partners in both games. For players that had met their partners beforehand, oxytocin increased their likelihood to cooperate, though this effect was very small for the prisoner's dilemma game. In essence, Declerck et al. showed not only that oxytocin likely requires social familiarity in order to have a prosocial effect but also that the absence of this familiarity renders oxytocin's effects antisocial. Moreover, oxytocin administration was not sufficient to significantly boost cooperativity among players in the prisoner's dilemma game, where it is not logical to choose

to cooperate. As others have cast similar results, “oxytocin makes people trusting, not gullible” (Mikolajcak et al., 2010).

Results such as these foreshadowed a body of literature that demonstrates an antisocial side to oxytocin (Zik and Roberts, 2015). In adult patients with borderline personality disorder, oxytocin administration has been shown to decrease the likelihood of trust and cooperation in a social dilemma game similar to the tasks described in the preceding paragraph (Bartz et al., 2011a; Ebert et al., 2013). In adults with trait physical aggressiveness, oxytocin administration was found to increase intimate partner violence inclinations (DeWall et al., 2014). Additionally, oxytocin has been shown to promote human ethnocentrism, a potential source of prejudice, xenophobia, and racial bias (Dreu and Greer, 2011; Sheng et al., 2013). Taken together, these data show that oxytocin can promote less than desirable behaviors especially for individuals with a propensity for maladaptive social responses. The significance of this pertaining to the use of oxytocin as a treatment in right hemisphere stroke is apparent. These data suggest that simple administration of oxytocin to patients is unlikely to produce the consistently prosocial effects that we would need from a viable treatment for social-emotional deficits in right hemisphere stroke. More than likely, oxytocin treatment will need to more carefully structure the environment under which oxytocin is administered to account for the full range of possible responses from prosocial to antisocial.

OXYTOCIN AND EMOTIONAL EMPATHY IN THE BRAIN

Thus far our discussion of oxytocin and empathy has been limited to a behavioral perspective, but imaging studies of healthy controls and patients with neurological disease have identified a set of key brain areas that are involved in emotional empathy and the oxytocin response. Three key areas in particular have emerged as a major sites of oxytocin action within the emotional empathy network: the insula, the temporal pole, and the amygdala.

The insula is an area of the brain known to be involved in perception of another person's emotions, especially negative emotions and pain (Singer et al., 2004; Chakrabarti et al., 2006; Gu et al., 2012; Bernhardt and Singer, 2012). In a group study of 27 patients, Leigh et al. (2013) found that lesions in this area were significantly associated with impaired performance on a test of affective perspective taking in acute stroke patients. Another study with a sample of combat veterans with focal traumatic brain injury similarly found a correlation between insular damage and decreased self-rated emotional empathy (Driscoll et al., 2012). In oxytocin imaging studies, it has been found that oxytocin increases activity in the insula in response to emotional stimuli such as infant crying and during tasks such as the RMET for patients with clinical depression (Riem et al., 2011; Pincus et al., 2010). Taken together, the data suggest that the insula plays a role in many of the component processes and damage to the region will likely detrimentally affect emotional empathy and social functioning as a whole (Hillis, 2014).

A second brain area identified as central to emotional empathy is the temporal pole. In a study mentioned earlier, Leigh et al. (2013) also found that all acute stroke patients tested that had damage in the right temporal pole had impaired empathy. Moreover, extent of the damage to the region was significantly correlated with errors in affective perspective taking. In

behavioral variant frontotemporal dementia, a neurological disorder characterized by disturbances in social functioning, the degeneration of right temporal pole has been found to be correlated to the level of social dysfunction observed (Rascovsky et al., 2011; Rankin et al., 2006). Functional imaging studies in healthy controls have corroborated these results as well showing that the temporal pole plays a role in affective perspective taking (Meyer et al., 2013). There is some evidence to suggest that oxytocin modulates activity in this region albeit more limited than the evidence connecting oxytocin to the insula and the amygdala (Domes et al., 2007; Domes et al., 2013).

The amygdala is the third area shown to be a major point of intersection between oxytocin and the emotional empathy network. Two activation likelihood estimation meta-analyses, one focused on empathic behavior and another focused on empathy for pain, both found that the amygdala was an area consistently associated with empathy (Bzdok et al., 2012; Gu et al., 2012). In the Leigh et al. (2013) study, acute stroke patients with lesions in the right amygdala showed impairment in affective perspective taking. Similar impairment was found in a previous study of two patients with acquired bilateral amygdala lesions (Stone et al., 2003). It is likely that the amygdala plays a role in both the affective and cognitive components of empathy with lesions leading to a major disruption in social cognitive functions and physiological manifestations of emotion (Hillis, 2014).

The amygdala is of particular interest when considering oxytocin's potential as a treatment for right hemisphere stroke. There is a robust body of evidence that oxytocin modulates amygdala activity; many of the imaging studies discussed in this chapter converge in support of this proposal (Domes et al., 2013; Bethlehem et al., 2012; Labuschagne et al., 2010; Riem et al., 2011). However, perhaps the most intriguing link between oxytocin and the amygdala is their relationship in socially reinforced learning. In a landmark double-blind study, Hurlemann et al. (2010) found that oxytocin administration increased learning performance on a feedback-guided item-category association task when social reinforcers were used as feedback. Moreover, they showed that this effect is amygdala dependent by demonstrating that two individuals with bilateral, congenital damage to the amygdala (Urbach-Wiethe disease) were not able to effectively learn through this pathway. This effect will require further study, but it does appear to have a basis at the synaptic level and the MAP kinase signaling cascade (Lin et al., 2012; Tomizawa et al., 2003).

ENVISIONING OXYTOCIN TREATMENT FOR RIGHT HEMISPHERE STROKE

To summarize, our goal is to explore the possibility of oxytocin treatment for social-emotional deficits in right hemisphere stroke patients that may involve issues with receiving, processing, and/or expressing affective or social information. The literature has established the context-dependence and individual factors that may influence the effects of oxytocin, and we have a few key brain areas that oxytocin is likely to modulate (Bartz et al., 2011b; Hillis, 2014).

From the very outset, while the vast majority of oxytocin experiments or treatment studies to date involve single doses. From these studies, the lasting benefits of single dose for patients with neuropsychiatric disorders with significant social-emotional impairments seem

questionable (Bartz, 2016). The loss of brain tissue due to stroke can cause severe deficits across a full spectrum of functions, requiring many months for recovery that is often incomplete (Frost et al., 2003). It is implausible that a single dose of a naturally occurring brain peptide hormone could lead to the dramatic neural reorganization needed to reverse damage caused by a large stroke. Nevertheless, treatment studies such as Jesso et al. (2011) in frontotemporal dementia patients should be taken as a proof of concept that warrant further study for long-term oxytocin treatment (Finger et al., 2014). To be truly useful in improving prognosis for stroke patients, oxytocin should be administered over the long term if it is to have an appreciable lasting effect.

This conclusion brings up an important consideration about the conditions under which oxytocin is administered and to whom it would be administered. As discussed earlier, oxytocin's effects are notoriously context and person dependent. If oxytocin is administered in an uncontrolled manner, it could induce antisocial behaviors that would be counterproductive to treatment aims. Moreover, it is possible that stroke patients may have preexisting psychiatric conditions such as borderline personality disorder that could cause oxytocin to worsen social functioning (Ebert et al., 2013). Many stroke patients develop depression during the recovery process. The potential interaction between depression and oxytocin will need to be further evaluated and considered (Hackett et al., 2005). In the context of stroke care, many patients stay in the hospital for extended periods and undergo inpatient rehabilitation for even longer periods. The best way to control the context under which oxytocin is administered and to monitor for adverse responses may be to integrate the treatment into these established clinical frameworks rather than allowing unmonitored use at home. The patient would likely need to feel supported and safe in order to observe the more positive effects seen in the Kosfeld era literature.

In the hospital setting, the effectiveness of oxytocin treatment could be increased if paired with another therapy such as social cognitive training. Trust is not built in a day and the pair-bonding effects of oxytocin are likely due to chronic exposure or elevation of central levels. The same could be said of social cognition and social functioning, with some authors proposing that social cognition be thought of as reinforced learning based on feedback from others (Zaki et al., 2016). In this view, social cognition is an adaptive process where we make inferences and then adjust them based on available feedback information. Social cognition is then less about the immediate accuracy emphasized in many tests (e.g., Reading The Mind In The Eyes Test) and more about a kind of adaptive accuracy. Given oxytocin's role in amygdala-dependent social learning, oxytocin may enhance this adaptive process through that pathway (Hurlemann et al., 2010). By encouraging this learning through social cognitive training, oxytocin may be able to facilitate recovery of social functioning more effectively. In other words, the amygdala-dependent learning pathway may be a vehicle to access plasticity that could enhance recovery of social function in right hemisphere stroke survivors. Given that the learned stimuli used by Hurlemann et al. were not explicitly emotional per se, it is likely that other abilities could be reinforced through oxytocin administration as well.

The benefits of oxytocin treatment may even extend beyond simply retraining social cognitive functions to strengthening the patient treatment alliance. Trust is an important component of the treatment alliance as it is critical that stroke patients trust their doctors and their caregivers. Social cognitive impairments may strain relationships between these three entities and decrease quality of care as a result. Notably, we have found that 50% of caregivers of right hemisphere stroke survivors cite empathic deficits as one their top five

concerns, which was more frequent than motor or sensory deficits (Hillis and Tippet, 2014). Given a positive social context, oxytocin may be able to address some of these empathic concerns and strengthen the trust between caregiver and patient (Meyer-Lindenberg et al., 2011; Lieberwirth & Wang, 2014). This effect of oxytocin on the treatment alliance has been observed in other clinical populations, which suggests that it may work in stroke as well. For instance, in a two-week pilot study of intranasal oxytocin for cocaine-dependent patients receiving methadone treatment, Stauffer et al. (2016) found that oxytocin caused patient reporting of drug use to more closely match the results obtained from urine testing, a result that the authors speculate could indicate increased trust. In individuals with post-traumatic stress disorder, it has been shown that oxytocin can normalize abnormal insula and putamen responses to social reward, which may stabilize social relationships in the treatment alliance over time (Nawijn et al., 2016).

CONSIDERATIONS FOR FUTURE OXYTOCIN TREATMENT RESEARCH

Two of the major concerns about current oxytocin research are statistical rigor and the method of delivery of intranasal oxytocin itself (Walum et al., 2016; Leng and Ludwig, 2015). Both of these articles argue for an increase in the statistical rigor of future oxytocin studies. At least some of the inconsistencies across studies can be explained by low power and inappropriate statistical methods in some studies, although some inconsistencies are likely due to context effects. Compliance with uniform standards, such as clearly defining outcome variables and specifying statistical analyses beforehand will no doubt increase the quality of future research. Healthy skepticism should be used when approaching oxytocin data, understanding that single dose effects in an experimental context should not necessarily generalize to a wide array of indiscriminate prosocial behaviors. Technical innovations may also be needed in the current method of delivering oxytocin intranasally, which to date is still not fully understood (Leng and Ludwig, 2015; Guastella et al., 2013). Large amounts are delivered via nasal sprays, but the mechanism and consistency of what occurs once the oxytocin reaches the nasal epithelium is unclear. Better methods of delivery may be needed to improve consistency in oxytocin trials.

Another important consideration for future oxytocin research in stroke patients is gender differences in oxytocin's effects. Much of the research covered in this chapter used samples primarily composed of males, and the effects may be different in women (e.g., Hurlemann et al., 2010; Kosfeld et al., 2005). Gender differences are likely given that the literature suggests that oxytocin's effects are indeed sexually dimorphic (MacDonald, 2013). Despite the strength of the evidence for a role of oxytocin in attachment in females, there are likely some undiscovered nuances (Bartz, 2016). Future research should account for individual differences as well as differences across sexes and ultimately adjust treatment accordingly.

CONCLUSION

Our understanding of oxytocin has advanced well beyond initial conceptions to include its significant context- and person-dependent effects. There still remains much work to be done before oxytocin will be ready to be integrated into standard practice in the clinic for use as a therapy in right hemisphere stroke patients. In particular, long-term trials will be needed to test the safety, efficacy, as well as the feasibility of using the treatment with social cognitive training, ideally during inpatient rehabilitation. Oxytocin presents considerable promise as a treatment option for stroke patients, but results will have to be considered with a new lens of nuance and systematic scientific rigor.

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BIOGRAPHICAL SKETCH

Name: Charlten Long

Charlten Long is a senior undergraduate student at Johns Hopkins University, where he majors in neuroscience. Charlten Long is currently a research assistant in Dr. Argye Hillis' Stroke Cognitive Outcomes Lab (S.C.O.R.E.), where he has worked for 3 years. As a recipient of a 4-year JHU Woodrow Wilson Undergraduate Research Fellowship, he established an innovative clinical trial, a collaborative study between JHMI and UCSF, aiming to use computerized training to treat social-emotional deficits in patients with right hemisphere stroke and frontotemporal dementia. Additionally, he contributes to an ongoing longitudinal fMRI study of language recovery after stroke. A native of California's Bay Area, Charlten previously worked in the labs of Dr. Sophia Vinogradov and Dr. Joshua Woolley. Working with Dr. Vinogradov, he received experience in researching the use of cognitive and social cognitive training in patients with schizophrenia. Charlten's work in Dr. Woolley's Bonding and Attunement in Neuropsychiatric Disorders Lab first exposed Charlten to the study of the effects of oxytocin administration for patients with schizophrenia. Charlten is interested in a future career of translational research searching for novel treatments to improve cognitive and functional impairments in patients with chronic neurological disorders.

Name: Argy E. Hillis

Argye E. Hillis is a Professor of Neurology, Physical Medicine & Rehabilitation, and Cognitive Science at Johns Hopkins. She serves as the Executive Vice Chair of Neurology, and Director of the Cerebrovascular Division. She began her career as a Speech-Language Pathologist and Director of Neurological Rehabilitation, focusing on studies of novel treatments of aphasia and communication disorders after right hemisphere stroke. She also studied Cognitive Neuropsychology in the Cognitive Science Department at Johns Hopkins, where she later became a faculty member. Her research focused on identifying the cognitive processes underlying language and spatial representations through the study of aphasia and hemispatial neglect, and how these investigations might help focus rehabilitation. Dr. Hillis then completed medical training and neurology residency at Johns Hopkins, and integrated her training in the fields of Speech-Language Pathology and Cognitive Science with Neurology to continue her investigations of aphasia and right hemisphere cognitive and

communicative impairments and how they recover. Her research combines longitudinal task-related and task-free functional imaging and structural imaging with detailed cognitive and language assessments to reveal the dynamic neural networks that underlie language and cognitive functions, such as empathy and prosody. Her lab studies changes from the acute stage of stroke through the first year of recovery, to improve our understanding how language and other cognitive functions recover after stroke and how to facilitate recovery. One study evaluates the effectiveness of transcranial direct current stimulation (tDCS) as a method of augmenting structured language therapy. The Hillis lab also studies Primary Progressive Aphasia, and how to reduce the rate of decline in language (with language therapy alone or augmented with tDCS). Her research is supported by the National Institute of Deafness and Communication Disorders and by the National Institute of Neurological Diseases and Stroke. She serves as Associate Editor of *Stroke and Aphasiology*, and is on several editorial boards.

Chapter 92

SHORT-TERM PREDICTORS OF MORTALITY AMONG PATIENTS WITH HEMORRHAGIC STROKE

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ABSTRACT

Background: Stroke is a major cause of disability and death. A significant number of patients with spontaneous intracranial hemorrhage (ICH) die within 30 days. **Objective:** To determine the mortality within 28 days in patients with hemorrhagic stroke.

Materials and methods: This case control hospital based study was carried out in the Department of Medicine of Rangpur Medical College Hospital, from 01 March, 2013 to 31st August 2013. The criterion of diagnosis was the presence of a hematoma in the brain by imaging.

Results: During the study span of 6 months, we studied over 110 patients, with a mean age of 56.02 years at the time of death. Mortality within 28 days was 45.5% (n = 50). Among those victims who died, hospital mortality was in 88% (n = 44) and approximately half of deaths (77.35%, n = 41) occurred within the first 7 days. Mortality was significantly higher among subjects with larger hematomas (>60 cm³) compared to

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subjects with smaller hematomas ($<30 \text{ cm}^3$; 68.9% vs. 22.9%, $P = 0.00008$). All stroke patients with ventricular extension, 67.9% ($n = 36$) died within 28 days. In the majority of cases (40.9%), lobar bleeding occurred and mortality was significantly higher in cases of lobar and cerebellar hemorrhage than deep hemorrhage. Glasgow Coma Scale (GCS) and volume of blood together also constituted a good predictor of mortality, as 90.90% (30) of the patients who had $\text{GCS} < 8$ and hemorrhage volume $> 60 \text{ cm}^3$ died within 28 days.

Conclusion: Short-term mortality (28 days) is very high in hemorrhagic stroke. GCS and volume of hematoma were independent predictors of mortality within 28 days.

Keywords: short term, hemorrhagic, mortality, Glasgow Coma Scale (GCS)

INTRODUCTION

Stroke is a major cause of disability and death. Approximately 16 million people worldwide are affected by stroke each year, and the estimated prevalence of stroke survivors is over 60 million [1]. In 2020, stroke will be the leading cause of death and disabilities after cancer throughout the world [2]. Numerous studies have been going on for the past 25-30 years, yet there is no uniform consensus regarding the most important predicting factors of mortality. To reduce the overall burden of stroke in society, an organized approach is needed to predict mortality and morbidity in stroke [3].

Clinicians are often asked to predict outcome after a stroke by the patient, family, nursing staff, therapy team, case managers, and insurance providers. A wide variety of factors influence stroke prognosis, including age, stroke severity, stroke mechanism, location of infarction or hemorrhage, co-morbid conditions, clinical findings, and related complications. However, knowledge of the important factors that affect prognosis is necessary for the clinician to make a reasonable prediction for individual patients, to provide a rational approach to patient management, and to help patient and family understand the course of the disease [4].

Brain imaging is the cornerstone for diagnosis of intracerebral hemorrhage (ICH). Although magnetic resonance imaging (MRI) is an excellent tool for considerable information on the process of acute stroke, MRI is not readily available to most patients who present with acute stroke to a rural or community hospital.

CT scan is the imaging modality of choice in patients presented with acute stroke, which can detect ICH within a few minutes of stroke onset [3]. It is safe and non-invasive and helps to measure the hematoma size, location of the hemorrhage and the presence of intraventricular, subarachnoid or subdural blood, or to find out any mass effect in primary non-traumatic ICH.

All this information is extremely useful in assessing the clinical and functional outcome in acute ICH, which cannot be obtained by clinical examination itself [5].

We have conducted this study to determine the short-term (28 days) mortality outcome of acute hemorrhagic stroke in patients admitted to the Department of Medicine of Rangpur Medical College Hospital.

METHODOLOGY

Populations and Methods

This case control study was carried out at the Department of Medicine, Rangpur Medical College Hospital. During the study span of 6 months, 9000 patients were admitted to the Department of Medicine, of which 902 presented with coma. Common causes of coma included stroke ($n = 4$), encephalitis ($n = 3$), hepatic encephalopathy ($n = 5$), uremic encephalopathy ($n = 4$) and hypoglycemia ($n = 4$), among others.

A convenient sampling method was used to include the study population. Patients presenting with spontaneous ICH were included for this study. The volume of ICH was calculated by the formula $ABC/2$, using the CT slice identified with the largest area of hemorrhage, where A is the largest diameter of the hemorrhage on this slice, B is the largest diameter perpendicular to A on the same slice, and C is the approximate number of 10-mm slices on which the ICH was seen. C was calculated by a comparison of each CT slice with hemorrhage to the CT slice with the largest hemorrhage on that scan. If the hemorrhage area for a particular slice was greater than 75% of the area seen on the slice where the hemorrhage was largest, the slice was considered one hemorrhage slice for determining C ; if the area was approximately 25% to 75% of the area, the slice was considered half a hemorrhage slice; and if the area was less than 25% of the largest hemorrhage, the slice was not considered a hemorrhage slice. These CT hemorrhage slice values were then added to determine the value of C .

All measurements for A and B were made with the use of the centimeter scale on the CT scan to the nearest 0.5 cm. A , B , and C were then multiplied and the product divided by 2, which yielded the volume of hemorrhage in cubic centimeters. The time required for the measurements and calculations was recorded. Hemorrhage volumes determined by this method were found to be in good agreement with measurements made by computer-assisted planimetric image analysis [6].

Procedure of Data Collection

An informed written consent was obtained from each patient or attendant before data collection. Each patient or attendant was interviewed. Besides socio-demographic characteristics GCS (Glasgow coma scale) at the time of admission, after 24 hours and at the time of discharge, blood pressure, neurological deficit, comorbidity, complications such as persistent neurological deficit, bed sores, aspiration pneumonia, urinary tract infection and painful shoulder were recorded. Short-term (28-day) mortality outcome was recorded as obtained by telephone calls to close relatives of the patients.

Procedure of Data Analysis

Data were coded, checked manually and entered into the computer. Data were analyzed using the SPSS-17.0 (Statistical Program for Social Science) software program. Clinical and statistical results are presented in Tables 1-7 and Figure 1.

Table 1. Socio-demographic characteristics of patients (n = 118)

Variables	Frequency	Percentage (%)
Age		
Mean age (SD)	56.02 years (SD $\pm$ 13.45)	
Age range	22-90 years	
Sex		
Male	53	48.2%
Female	57	51.8%
Level of education		
Illiterate	57	51.8%
5 or less class	35	31.8%
>5-10 class	7	6.4%
>10-12 class	2	1.8%
Graduate and above	9	8.2%
Occupation		
Housewife	55	50%
Agriculture	22	20%
Business	13	11.8%
Service	8	7.3%
Retired	4	3.6%
Others	8	7.3%
Monthly income		
<5000 taka*	54	49.1%
5001-10000 taka	27	24.5%
10001-15000 taka	18	16.4%
>15000 taka	11	10%

*1 USD=80 taka.

Of the study participants, 23.6% (26) were current smokers, 10.9% (12) were ex-smokers and 23.6% (26) were sedentary workers. Among the women, 68.42% (39) used different contraceptives (Table 2).

Table 2. Frequency of use of different types of contraceptives (n = 39)

Contraceptive	Frequency	Percentage
Oral	32	56.1%
Injectable contraceptive	2	3.5%
Others (barrier method, biological method)	5	8.8%

Table 3. Co-morbidity of the study population (n = 110)

Co-morbidity	Frequency	Percentage
Hypertension	66	60%
Diabetes mellitus	1	0.9%%
Hypertension and diabetes	3	2.7%
Hypertension, diabetes and dyslipidaemia	1	0.9%
Hypertension and dyslipidaemia	2	1.8%
Hypertension and CKD	3	2.7%
Dyslipidaemia	3	2.7%
Ischaemic heart disease	2	1.8%
No	29	26.2%

Table 4. Site of bleeding among study population

Site of bleeding	Percentage	Mortality at 28 th day
Lobar	40.9% (45)	64.4% (29)
Cerebellum	5.5% (6)	66.7% (4)
Deep hemorrhage (Thalamus and basal ganglia)	39.1% (43)	20.9% (9)
Pons	6.4% (7)	57.1% (4)
Subarachnoid	3.6% (4)	25% (1)
Multiple sites	4.54% (5)	60% (3)

68.18% (75) of the study patients had hypertension and 5.45% (6) had diabetes mellitus (Table 3). A family history of hypertension was present in 55.5% (61) of the patients; among them, hypertension was present in 83.33% (50), and a family history of stroke was present in 24.5% (27). In the majority (40.9%) of cases lobar bleeding occurred and bleeding at multiple sites occurred in 4.54% (5) (Table 4). Different complications following the stroke occurred in 49.1% of the patients. Among the complications, chest infection was the most frequent (29.6%) (Table 5).

Table 5. Complications after intracranial hemorrhage

Complications	Frequency (n = 54)	Percentage
Chest infection	18	33.33%
Bed sore	13	24.07%
Seizure	10	18.51%
Painful shoulder	7	12.96%
Electrolyte imbalance	4	7.40%
Others	2	3.70%

Operational Definitions

Hemorrhagic stroke: Patients presented with sudden onset of focal neurological deficit (e.g., sudden loss of consciousness, aphasia, weakness of one side of the body, sudden loss of vision) and CT/MRI evidence of intracerebral hemorrhage was labeled as hemorrhagic stroke.

Sites of cerebral hemorrhage: The main sites of cerebral hemorrhage are the thalamus, putamen, pons, cerebellum and temporal lobes [7].

Short-term outcome of acute stroke: In our study, the outcome 28 days after onset was taken as short-term outcome (death or survival). Patients alive on day 28 were considered to have survived.

Smoking

Those who currently smoked or had smoked tobacco in any form (cigarette, birri, etc.) in the last 6 months were considered to be smokers. Ex-smokers were patients who gave up smoking at least 6 months before stroke onset.

Smokeless Tobacco (SLT)

SLT consists of raw tobacco leaves not consumed by inhalation (usually taken with betel nut or held between the cheek and jaw).

Statistical Analysis

Socio-demographic data were compared between patients who died and those who survived by one-sample Student 't' test. Their observed differences, expressed as frequency distributions, were tested by the 'chi-square' test. A P-value of <0.05 was considered as statistically significant and 95% confidence intervals were computed.

Ethical Consideration

The ethical committee of Rangpur Medical College approved the study protocol and questionnaire.

RESULTS

During the study span, we examined over 110 patients. The mean age of the patients at the time of death or discharge was 56.02 years. There were slightly more females than males (51.8% vs. 48.2%). The majority of the patients (87.3%) came from rural areas. Table 1 shows the socio-demographic characteristics of the study population.

Mortality within 28 days for patients with intracerebral hemorrhage was 45.5% (50). Among these, hospital mortality occurred in 88% (44). 28-day mortality was slightly higher in females than in males (54% vs. 46%) and the mean age at death was 56.46 years. Most patients who died (62%) were over 50 years of age. Death was more common (65%) in older patients (>70 years), in smokers (57.7%) and in those who had hypertension (54.7%).

Among the ICH patients, volume of blood was >60 cm³ in 40.9%, 30-60 cm³ in 27.3% and <30 cm³ in 31.8%. Mortality was significantly higher in those whose volume of blood was >60 cm³ than those whose volume of blood was <30 cm³ (68.9% vs. 22.9%) (P = 0.0000844) (Table 6).

More than half of the deaths (77.35%; 41) occurred within the first 7 days. 82.35% of the patients who had a volume of blood >60 cm³ died within 7 days, compared with 50% of patients whose blood volume was <30 cm³ (P = 0.00002209). 48.2% (53) of the patients had intracerebral hemorrhage with ventricular extension; 67.9% (36) of them died within the first 28 days and 80.55% (29) of the deaths occurred by the seventh day.

The mean GCS of the study population at the time of admission was 9.06; after 24 hours 9.40 and at the time of discharge 13.27. 88% (44) of the patients who had a GCS of <8 at presentation died within 28 days, and the majority (90.90%) of deaths occurred in the hospital. Only 10% (6) of the patients who had an initial GCS > 8 died within 28 days. A significant improvement of GCS occurred from presentation (GCS = 11.47) to day 28 (14.46) in patients who survived. No significant improvement occurred from presentation (GCS = 6.10) to 24 hours later (6.12) in patients who died (Figure I).

GCS and volume of blood together also constitute a good predictor of mortality within 28 days. The majority (90.90%) of the patients who had GCS<8 and a hemorrhage volume >60cm³ died within 28 days (Table 7).

Among the study population, GCS <8 and ventricular extension were present in 35.46% (39) of patients, and 84.61% (33) of them died within 28 days.

Table 6. Relation of mortality with volume of intracerebral hemorrhage

Volume of blood	Percentage	Mortality at 28 th day
<30 cm ³	31.8% (35)	22.9% (8)
30-60 cm ³	27.3% (30)	36.7% (11)
>60 cm ³	40.9% (45)	68.9% (31)

Table 7. Relation of mortality with GCS and volume of hemorrhage

GCS	Volume of hemorrhage	Percentage/frequency	Mortality
<8	>60cm ³	66% (33)	90.90% (30)
	30-60 cm ³	22% (11)	72.72%% (8)
	<30 cm ³	12% (6)	100% (6)

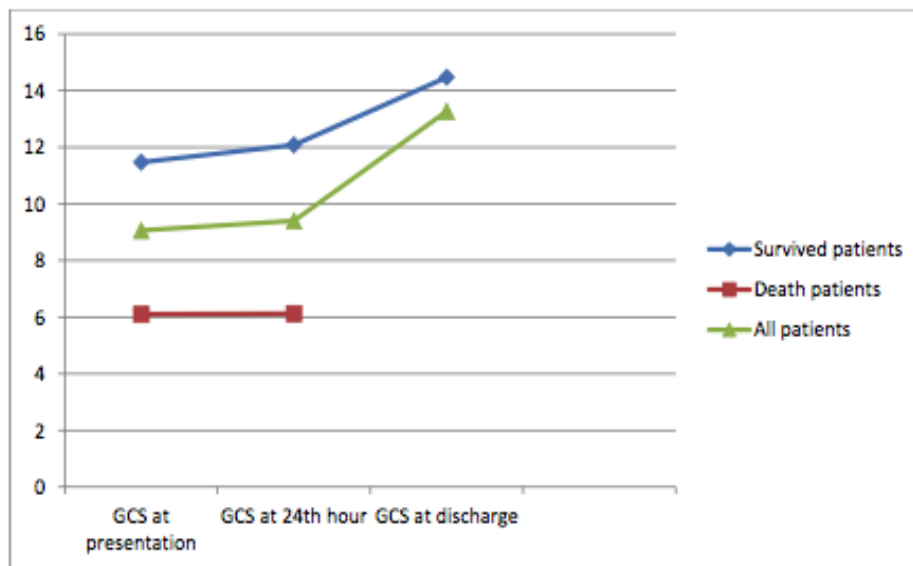


Figure 1. Time course of GCS in all patients and in patients who died or survived.

DISCUSSION

In the acute phase of stroke, the main predictors of outcome are stroke severity and patient age. Stroke severity is probably the most important factor affecting short- and long-term outcome [8-18]. Intracerebral hemorrhage has a reported 30-day mortality of 42% [19].

Our study found near similar findings at 28-day mortality after spontaneous intracerebral hemorrhage (45.5%). In our study, the majority (77.35%) of deaths occurred by day 7, with more than half of the deaths occurring within 5 days. This finding differs from other studies reporting that more than half of the deaths occurred by the end of the second day [20-21]. Factors independently associated with 30-day mortality were hemorrhage volume, level of consciousness, and presence of intraventricular extension [22].

Different techniques have been developed to measure hemorrhage volume [23-26]. Unfortunately, these methods often involve complicated formulas, require specialized equipment, or cannot be performed rapidly at the patient's bedside.

Broderick [29] showed that the simple formula $ABC/2$ can accurately estimate intraparenchymal hemorrhage volume and requires less than 1 minute for measurement and calculation. The measurements correlate highly with the volumes calculated by planimetric methods for all hemorrhage locations. Rapid calculation of ICH volume at the time of initial patient presentation has clinical utility. For prognosis, a model of 30-day mortality using the Glasgow Coma Scale and hemorrhage volume in patients with ICH correctly predicted outcome with a sensitivity and specificity of 97% [27].

In our study, hemorrhage volume was a strong predictor of short-term mortality. Mortality by day 28 was higher in large hemorrhages ($>60 \text{ cm}^3$) in comparison to small hemorrhages ($<30 \text{ cm}^3$) ($P = 0.0000844$). Large-volume hemorrhage is also a good predictor of mortality within 7 days of onset of intracranial hemorrhage (82.35% vs. 50%) ($P =$

0.00002209). Stroke severity on admission depends on the level of consciousness and was the main clinical predictor of early mortality in many previous studies [28-32]. Level of consciousness can be easily determined at the bedside by the GCS. Our present study showed that GCS at presentation was a good predictor of mortality during the hospital stay and within 28 days. GCS after 24 hours is also a good predictor of improvement or deterioration in ICH patients. GCS and volume of blood together are also a good predictor of mortality within 28 days. Analysis of subgroups showed that 90.90% of the patients with GCS <8 and hemorrhage volume $>60\text{cm}^3$ died within 28 days. But 100% of the patients with GCS <8 and hemorrhage volume $<30\text{cm}^3$ also died within 28 days. Thus, GCS alone is a more reliable predictor of short-term mortality than hemorrhage volume alone. Apart from GCS and volume of hemorrhage, other factors such as site of hemorrhage and complications after stroke may be important determinants of death. In our study, ICH with intraventricular extension was an important predictor of mortality within 28 days ($P = 0.0018$); intraventricular extension along with GCS <8 was also a very important predictor: 84.61% of patients with ICH with intraventricular extension with GCS <8 died within 28 days.

Sukdeb et al. [33] showed that mortality was higher in patients with lobar hemorrhage (40%) than with subcortical hemorrhage (27%); this result is consistent with our findings. In our study, mortality in patients with lobar hemorrhage was 64.4% and in those with deep hemorrhage it was 20.93%. In cerebellar hemorrhage, mortality was found to be 4% [34] and 40% [33] in other studies compared to 66.7% in our study. High mortality may be due to poor resources of surgical evacuation in cerebellar hemorrhage. Advancing age has a major negative impact on stroke morbidity, mortality, and long-term outcome [35-39].

In our study, there was no significant difference of mortality in older (>60 years) or younger (<60 years) patients ($P = 0.57160764$). Data regarding sex differences and stroke outcome are conflicting. In our study, though mortality was slightly higher among females than among males (47.4% vs. 43.4%), the difference was not statistically significant ($P = 0.57160764$).

This study is important because hypertension and stroke in Asia have become highly prevalent, without any strategies for control or prevention [40].

CONCLUSION

Short-term mortality (28 days) is very high in hemorrhagic stroke. ICH with intraventricular extension, GCS and volume of blood independently and together are good predictors of mortality within 28 days.

Limitation

Sample size was small and other factors that may contribute to mortality, such as electrolyte disturbances and infections, were not addressed.

Future Direction

Study with large sample size will be needed to clarify the predictors of mortality in hemorrhagic stroke patients.

Conflict of Interest

There was no conflict of interest.

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Contributions by the Authors

Dr. Ratindra Nath Mondal planned and supervised the study. Dr. Md. Jahedul Islam, Dr. Md. Ashraful Haque, ABM Mobasher Alam analysed the data. Dr. Shah Md.Sarwer Jahan, Dr. Md. Mahfuzer Rahman, Dr. Md. Kumruzzaman Sarker, Dr. Moni Rani, Muhammad Mahbub Hussain, Haripada Sarkar helped to collect the data. Dr. B. D. Bidhu, Professor Dr. Md. Zakir Hossain, Professor Dr. Amaresh Chandra Shaha, Professor Ram B. Singh, Professor Dr. Md. Noor Islam helped in writing of the manuscript and presentation of the data.

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Chapter 93

ELECTRICAL STIMULATION IN STROKE PATIENTS

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ABSTRACT

Surface electrical stimulation is one of the tools suggested for the rehabilitation treatment of patients following a stroke. Although its use is not very widespread in current clinical practice, it is regarded as a non-invasive, low-cost, reliable and easy-to-use treatment which could stimulate recovery in stroke patients. In recent years, the understanding of motor learning, neuronal plasticity and functional recovery after brain damage has improved, and the new findings in neuroscience have given a fresh impetus to research in neurorehabilitation. The number of existing studies regarding electrical stimulation and the stroke patient shows the interest which the subject still provokes and the need to provide more evidence that will assure its beneficial effect in clinical practice. Current lines of investigation are principally focussed on the recovery of the upper-limb, lower-limb and gait, hemiplegic shoulder, dysphagia, urinary incontinence and visuo-spatial neglect. Additionally, different modalities of application are presented and the most effective protocols are investigated, including their complementary use with other therapeutic strategies and new technologies. In this chapter, these points are extensively discussed to showcase the latest evidence, shed more light on this intervention in the field of neurological rehabilitation and establish future lines of interest in which advances in neuroscience and technological development play a significant role.

Keywords: surface electrical stimulation, stroke, neurorehabilitation

INTRODUCTION

Neuromuscular electrical stimulation (NMES) involves the use of electrical current to stimulate neural and muscular structures (Rodríguez, 2004). Although NMES can be applied

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using superficial, percutaneous or implanted electrodes (IJzerman et al., 2009), in this chapter we will further explore the superficial electrical stimulation as a therapeutic strategy, presented as a non-invasive (Díaz et al., 2005), low-cost, reliable and easy-to-use treatment (Glanz et al., 1996; Sentandreu et al., 2011). NMES has been applied for different purposes and in different pathologies. Among such pathologies, it has been suggested for the treatment of patients following a stroke (Howlett et al., 2015).

A stroke is a sudden, non-convulsive loss of neurological function due to an ischemic or haemorrhagic intracranial vascular event (Mehrholtz et al., 2013). It is the main cause of adult disability and the third most common cause of death in Europe (Scutt et al., 2015). The incidence of stroke increases with age (Rigau et al., 2009) and due to the progressive aging of the population more health care requirements are expected in the case of these patients (Bermejo et al., 2009). In addition to disturbances in the cognitive and affective domains, the loss of motor function represents the most serious condition after a stroke (Soekadar et al., 2015). In this respect, the rehabilitation of patients after a stroke constitutes a worldwide challenge (Liu et al., 2016) and innovative methodologies for the purpose of restorative neurorehabilitation are urgently required in order to reduce long-term disability (Dutta et al., 2014).

The first paper reporting on the use of NMES in neurological patients was published in 1961. Liberson et al. (1961) described the use of electrical current to stimulate the dorsal flexors of the foot in stroke patients to prevent drop-foot during the swing phase of gait. Since then, the understanding of motor learning, neuronal plasticity and functional recovery following brain damage has improved and the new findings in neuroscience have given a new impetus to research in neurorehabilitation. This improved understanding has led to the identification of new clinical roles, challenges and emerging research in the field of NMES and stroke patients.

The vast number of existing studies concerning this subject is indicative of an ongoing interest and an increasing need to provide further evidence that will ensure the beneficial effect thereof in clinical practice. Current lines of investigation are principally focussed on the recovery of the upper-limb, lower-limb and gait, hemiplegic shoulder, dysphagia, urinary incontinence and visuo-spatial neglect; different modalities of application are presented and the most effective protocols are investigated, including their complementary use with other therapeutic strategies and new technologies. These points are discussed in greater detail within the framework of this chapter to show the most recent evidence, shed more light on this intervention in the field of neurological rehabilitation and set out future lines of interest in which advances in neuroscience and technological development play a significant role.

UPPER-LIMB

Impairment of the upper limb is one of the most frequent consequences of stroke (Mangold et al., 2009) and directly impacts on the patient's function and quality of life. It is estimated that less than 50% of stroke survivors will recover arm function (Nakayama et al., 1994), which will exert a great economic, social and personal toll. Among the different therapeutic tools, NMES has been proposed in several studies as a strategy in the rehabilitation of the upper limb of stroke patients, and different reviews and meta-analyses

support its use (Howlett et al., 2015; Nascimento et al., 2014). However, despite of it, this topic remains at present a research focus for various authors as can be seen with the number of studies in the literature.

The most recent research on electrical stimulation and upper limb recovery focuses mainly on the most effective protocols by comparing different variables of the applied programmes (i.e., NMES modalities, stimulation parameters, electrode placement, etc.), the characteristics of the patients who can benefit most from this treatment, its effectiveness as a complement to other emerging therapies, and its effects not only locally but also its impact on the central nervous system.

Regarding the main NMES modalities found in the randomised controlled trials (RCTs) reviewed, certain studies have applied sensory or motor electrical stimulation thresholds. In the first approach, the stimulation evokes a sensory reaction without muscle contraction. Some authors also include the transcutaneous electrical nerve stimulation (TENS) modality in this group. In the second approach, where a muscular contraction is provoked, several applications can be distinguished: cyclic NMES (without voluntary activity by the patient), sensor or electromyographic (EMG)-triggered NMES (activated voluntarily by the patient) and functional electrical stimulation (FES) (associated with the performance of functional tasks). In some of these cases, NMES treatment was applied by orthosis. One of the most used models in the studies reviewed is the NESS H200 surface FES system for upper limbs (Bioness Inc., Santa Clarita, CA, USA). This model combines wrist-hand orthosis with muscle activation of the paralyzed forearm and hand via integrated surface electrodes. It allows consistent replication of the grasp, hold and release functions of the hand.

Few previous studies have directly compared electrical stimulation modalities, and although many modalities have been established, conclusive proof of their effects is still lacking (Hara et al., 2008). De Kroon et al. (2008) and Boyaci et al. (2013) compared cyclic NMES and EMG-triggered NMES, and found no difference in outcomes. These findings disagree with the review by Kroon et al. (2005), which argued that electrical stimulation triggered by voluntary movement appeared to be more beneficial than other NMES modes. It has been hypothesized that the intention to execute a movement, along with peripheral activity, reinforces plastic changes further compared to cyclic stimulation (Quandt and Hummel, 2014). A recently published study (Wilson et al., 2016) that compared the effect of cyclic NMES, EMG-triggered NMES and sensory stimulation, concluded that all groups exhibited significant improvement of impairment and functional limitation in the upper limb, though there was no difference based on the type of electrical stimulation administered.

Electrogoniometers, bend sensors, touch sensitive mats, and accelerometers are among the external sensors that have been incorporated into NMES systems (Knutson et al., 2015). Researchers also continue to explore the use of signal collection from the impaired upper limb to not merely trigger the onset of a preset intensity and duration of stimulation but also to control the intensity and timing of stimulation. A challenge for EMG-triggered NMES modalities is that the effort required from patients to contract the muscle does not develop flexor synergies or hypertonia (Knutson et al., 2015).

Contralaterally controlled functional electrical stimulation (CCFES) is an innovative method of sensor-controlled stimulation that uses movement from the unimpaired side to control the timing and intensity of stimulation to the paretic side. The hand system consists of a glove with bend sensors worn on the non-paretic hand and a multi-channel stimulator that delivers stimulation to the paretic hand extensors with an intensity that is proportional to the

degree of opening of the glove. A recent study indicated that CCFES improved hand dexterity more than cyclic NMES in chronic stroke patients (Knutson et al., 2016).

Recent advances in neurotechnology have led to the development of brain-computer interfaces (BCIs) that translate brain activity into control signals of computers (Venkatakrisnan et al., 2014). This technology provides a means to decode mental states and activate devices according to user intentions, and could provide a direct feedback on the engagement of motor areas of the brain surrounding the lesion site (Millán et al., 2010). BCI combined with output devices such as FES has also been studied (Silvani et al., 2011). BCI with a FES system has been explored as a potential rehabilitation tool for motor learning after stroke because FES can assist such patients in learning and practicing the desired coordinated movements (Müller-Putz et al., 2005). Biasiucci et al. (2013) reported preliminary results of a BCI with a FES system for stroke rehabilitation where the user's intention to perform an extension movement of the affected hand was detected through a BCI and used to activate a FES device. These results confirmed the beneficial effects of direct muscle stimulation according to user intentions to perform a motor task. Along the same line, Daly et al. (2009) carried out a case study which demonstrated the feasibility of combining BCI and FES for motor learning in stroke patients. After training sessions, volitional control of isolated finger extension was obtained. These results showed that combining BCI and FES promoted motor recovery. The authors argued that this improvement could be attributed to BCI, FES, specific target-oriented physical practice or a combination thereof. In this way, RCTs are required to determine whether BCI actually can have an additive effect beyond FES alone and to recognise the characteristics of stroke patients who can successfully use BCI as a motor learning paradigm. On the other hand, Kim et al. (2016) investigated the effects of action observational training (AOT) plus BCI-based FES on improving upper arm function in stroke patients. Their findings suggest that AOT plus BCI-based FES has a positive influence on upper extremity performance by improving functional mobility and ROM of paretic stroke patients.

Specific stimulation parameters are the subject of debate (Quandt and Hummel, 2014). As is also pointed out in other reviews, the comparison of stimulation parameters is complicated due to the disparity of variables or the omission of data. It has been mentioned that neurophysiological responses vary according to the parameters used, and though their clinical relevance has not been verified, even they may not be crucial in determining the effect of electrical stimulation (de Kroon et al., 2005). The most commonly used were low frequency rectangular biphasic currents, with frequency values of 20 and 100 Hz. The intensity was adapted to the sensory or motor threshold, the pulse duration varied between 100 and 500 μ s, and duty cycles as well as ramping up/down periods presented different combinations, in addition to treatment doses. Cauraugh et al. (2003) compared protocols with different times of contraction, concluding that better results were obtained with stimuli of 10 s. Doucet and Griffin (2013) compared protocols with different frequencies and concluded that the effects differ depending on the frequency applied. Sentandreu et al. are currently finalizing a study where two experimental groups with different stimulus frequencies and a control group with conventional therapy have been compared. Their results will be published soon. Clair-Augier et al. (2012) investigated the effect of pulse duration on the paretic and non-paretic arm and found a significant increase in elbow flexion torque for the former when using a wide-pulse stimulation scheme (1 ms). It was speculated that wide-pulse stimulation may lead to a greater reflexive recruitment in the paretic arm, highlighting that pathological

changes influence stimulation response. Conforto et al. (2010) concluded that sessions of repetitive peripheral nerve sensory stimulation (PSS) could facilitate motor function after subacute stroke depending on the intensity of the stimulation used. The authors proposed that careful dose-response studies are needed to optimise parameters of PSS before designing costly, larger, double-blind, multi-centre clinical trials.

Regarding treatment doses, in the meta-analysis of Pomeroy et al. (2006), it was pointed out that the length of time that NMES is applied could influence its effectiveness. Some studies have also remarked that one of the reasons for the lack of effectiveness of some programmes could be due to the short duration of treatment (Rosewilliam et al., 2012; Popovic et al., 2002), as well as the need for longer protocols in patients with severe impairments (Mangold et al., 2009; Alon et al., 2008; Popovic et al., 2003). Page et al. (2012) compared the efficacy of different rehabilitation protocols in subacute stroke patients and found that 120 minutes of repetitive task-specific practice combined with electrical stimulation was superior compared to shorter durations of stimulation. In contrast, Hsu et al. (2010) argued that higher and lower doses of NMES led to similar improvements in motor function. A minimum of 10 hours of NMES in combination with regular rehabilitation may improve recovery of arm function in acute stroke patients with severe motor deficit. Along the same line, Kowalczewski et al. (2007) compared two protocols of high and low intensity and only some measures obtained significant changes. Lourenção et al. (2005) reported that six months of FES were necessary for a significant improvement in grip speed, and it was hypothesized that five months would be necessary for an effective programme to improve upper limb function.

As for the electrode placement and stimulated musculature, there is also a diversity of options. Some studies have applied NMES at a unique segmental level in agonist and/or antagonist musculature, and others have made a multi-segmental application, applying electrical stimulation in several segments of the upper limb. There is evidence that greater effect sizes are associated with movements that stimulate functionally useful movements, as seen in studies that stimulate more than one joint (Farmer et al., 2014) such as that by Meadmore et al. (2014). They applied FES to three muscle groups in the upper limb and completed coordinated, repetitive, goal-oriented movements using real objects, controlling the FES signal to each muscle using advanced iterative learning control algorithms in order to ensure that the resulting movement precisely coincided with unimpaired task competition. The positive results indicated that multiple muscle stimulation along with the application of iterative learning technology is promising in stroke rehabilitation and may prove effective in reducing upper limb impairments. De Kroon et al. (2004) compared the stimulation of extensors and flexor-extensors and found no difference. Hara et al. (2007) argued that the combined use of distal EMG-NMES and proximal stimulation (anterior deltoid and triceps brachii) could be more efficient on a functional level.

Concerning patient characteristics, some studies have pointed out the importance of suitable sample selection (Powell et al., 1999; Sentandreu et al., 2011). It has also been claimed that motivated patients (Kroon et al., 2005) with moderate impairment (Powell et al., 1999) and residual motor function (Popovic et al., 2003; Powell et al., 1999) could benefit from NMES. According to this, Chae et al. (2008) added that mild to moderately impaired subjects could benefit most.

Recent studies have investigated NMES combined with other emerging physical therapy approaches and new technologies such as mirror therapy, mental imagery training, bilateral

movement training, constraint-induced movement therapy (Page et al., 2006) and robotics. Superior effects were shown in studies employing protocols where electrical stimulation was combined with the mirror therapy (Kim et al., 2015; Lin et al., 2014; Yun et al., 2011). Hong et al. (2012) combined EMG-triggered FES with mental imagery training and observed an advantage over FES alone. In contrast, Hemmen et al. (2007) investigated the effect of movement imagery combined with EMG-triggered NMES compared with a conventional NMES programme and concluded that there was no difference between the two therapeutic protocols. With regard to bilateral movement training, on the one hand, Cauraugh et al. (2002, 2005) concluded that the results were more prevalent in groups where EMG-triggered NMES was applied along with bilateral movement training. Knutson et al. (2007) as we have described before, also obtained more benefits in CCFES compared with cyclic NMES. This modality also combines the effects of NMES, active and goal-oriented training, and bimanual symmetric exercises involving the unaffected upper limb in the training of the affected upper limb. On the other hand, Singer et al. (2013) did not show that a protocol applying bilateral tasks plus EMG-triggered NMES was superior to the unilateral equivalent.

With regard to robotic systems, there is a paucity of information regarding the comparative benefit of NMES (McCabe et al., 2015). O'Connor et al. (2015) investigated the feasibility of using the iPAM Mk2 dual robot rehabilitation system with NMES to provide simultaneous hand opening exercises during assisted reaching exercises for stroke survivors who have moderate to severe arm impairment. Based on the promising findings in this report, the authors suggested that future work be planned to explore the possibilities of further combinations, including in lower limb rehabilitation, and to optimise the characteristics of robotic and stimulation parameters. Hu et al. (2015) concluded that NMES robot-assisted wrist training was more effective than the pure robot training. The additional NMES application in the treatment could result in more improvements in distal motor functions and faster rehabilitation progress. McCabe et al. (2015) compared the response to upper-limb treatment using robotics plus motor learning (ML), FES plus ML and ML alone, in a group of chronic severely impaired stroke survivors. The authors did not find any difference in treatment response across the three intervention groups according to the measures of joint movement coordination or complex functional task performance. On the other hand, Daly et al. (2005) pointed out that comparison of NMES and robotics resulted in different outcomes depending on the therapy applied.

Despite the increased knowledge regarding neural plasticity and brain mapping studies, the specific mechanisms of action of these different electrical stimulation modalities are not clear. It has been speculated that therapeutic effects are probably caused by a combination of local and central effects. At the local level, reference has been made to changes in muscular strength, modification of viscoelastic characteristics and increase of blood flow, among others (de Kroon et al., 2004; Ring et al., 2005). Quandt and Hummel (2014) argued that NMES could influence cortical reorganization. It is hypothesized that either concurrent stimulation of afferent fibres, or antidromic stimulation lead to enhanced synaptic remodelling, but evidence is still lacking. Hara et al. (2008) suggested that sensory components of large afferent fibre activation, proprioceptive input, and increased cognitive sensory attention could enhance voluntary movement and function. NMES is associated with concomitant physiologic changes in the brain including activation of primary sensory and motor areas and the supplementary motor area, reduction of intracortical inhibition, and increased amplitude of motor-evoked potentials (Chae et al., 2008). Currently, with access to advanced neuroimaging

tests, many studies are aimed at investigating the changes that NMES causes at the level of the central nervous system. Several studies demonstrated evidence of central mechanisms using neurophysiologic experiments such as reaction time and fMRI (Chae et al., 2008). Kimberley et al. (2004) indicated that the NMES programme resulted in improvements in the functionality of the hand and these were associated with an increase in cortical intensity in the ipsilateral primary sensory cortex. According to these authors, Quandt and Hummel (2014) argued that most of the reviewed studies found changes of neural activation after NMES use and a trend towards severe impairment leading to activation of the contralesional sensorimotor cortex can be observed. On the other hand, less impaired patients tend to recruit the ipsilesional site. The literature of cortical reorganization during stroke recovery, describing the relationship of lateralization and functional outcome, supports these conclusions.

LOWER-LIMB AND GAIT

Post-stroke gait dysfunction is among the most investigated neurological gait disorders and one of the principal goals of post-stroke rehabilitation (Lindquist et al., 2007). At the level of the lower limb, paresis, along with the inability to grade muscle contractions, poor motor coordination, poor endurance, spasticity, and impaired balance have significant consequences on ambulation (Eng et al., 2007). In particular, the inability to dorsiflex the ankle during the swing phase of gait affects approximately 30% of patients following a stroke, which increases the risk of falls and mortality (Bosch et al., 2014). These impairments have an important impact in patient's life and entail considerable costs for health and social services (Evers et al., 2004).

With reference to the electrical stimulation modalities, NMES to the lower extremities is mainly used by post-stroke patients as an alternative to mechanical orthotic devices during gait or as a training modality during rehabilitation (Kafri et al., 2015). In the second approach, the same modalities described in the upper limb may be observed. Nevertheless, the most widely used alternative is FES, which is associated with the performance of functional activities.

In 1961, Liberson et al. (1961) used FES as a substitute for traditional drop-foot correction within a hemiplegic population. By triggering a foot switch, a single-channel electrical stimulation device stimulated the common fibular nerve via a surface electrode, resulting in dorsiflexion during the swing phase of gait. This led to the first commercially available surface electrode stimulator. Since then, advances in technology and the development of wireless FES systems for the purpose of drop-foot correction have become commercially available and are used as an alternative to the ankle-foot orthosis (AFO) (Kafri et al., 2015). A trigger is used to stimulate the muscles during the specific phases of gait. The most common trigger is a foot switch, which is activated by the user's weight upon initial contact. The dorsiflexion can also be stimulated via the use of a tibial tilt sensor capable of detecting the tibial position during the terminal stance (Bosch et al., 2014). Recently, several devices have been commercially introduced for drop-foot correction. Relevant examples within this context would be the WalkAid® (Hanger Orthopaedic group), a surface peroneal nerve stimulator controlled by a tilt sensor attached just below the knee; the NESS L300™, a

new-generation surface electrical stimulation system for drop-foot controlled by a heel switch that wirelessly communicates with the stimulator integrated within a semi-rigid orthosis positioned just below the knee; and the Odstock Dropped Foot Stimulator, which represents one of the most widely used and best documented surface stimulation systems. A portable gait event detection device is essential for ensuring the appropriate timing of the stimulation. A variety of sensors, such as goniometers, gyroscopes, accelerometers, inclinometers or force sensitive resistors and various sensor combinations have been developed in order to meet these requirements (Kafri et al., 2015).

As was described in the upper limb section, CCFES has also been used to improve recovery of ankle dorsiflexion in chronic and subacute stroke patients. Initial studies provided preliminary evidence in support of the efficacy thereof (Knutson et al., 2012).

There has been a growing interest in employing BCI technology in combination with FES in post-stroke gait therapy (McCrimmon et al., 2015). Chung et al. (2015) conducted a pilot study in order to determine the BCI-FES effects on balance and gait function in patients with stroke. The results of this study suggested that BCI-FES training constituted a more effective exercise for the amelioration of balance and gait function as opposed to FES training alone in patients with stroke. The authors argued that further studies are needed to investigate its long-term intervention effects. In addition, McCrimmon et al. (2015) explored the safety and feasibility of a foot-drop-targeted BCI-FES physiotherapy in chronic stroke survivors. The authors concluded that BCI-FES therapy was safe and that a large number of subjects experienced improvements in terms of gait speed and dorsiflexion aROM.

Optimal treatment protocols for the lower limb and gait recovery are difficult to elicit due to the inconsistent and wide ranging outcome measures, varying modalities to NMES, and the different stimulation parameters used (Kafri et al., 2015). Kafri et al. (2015) reported that in the case of reviewed studies, frequency values generally ranged between 15-50 Hz, whereas pulse duration varied between 280-400 μ s in line with a current amplitude value of 4-85 mA. The authors indicated that the stimulation parameters were not reported in a consistent manner, stating that while some studies did not report any parameters, others varied in the amount of detail provided. Kesar et al. (2010) compared knee and ankle kinematics during the swing phase of gait when FES was delivered to the ankle dorsiflexors using variable frequency trains (VFTs) versus constant-frequency trains (CFTs). A 30 Hz constant-frequency train was applied in the case of FES using CFTs, whereas a 3-pulse burst of high frequency (200 Hz) followed by a lower frequency (30 Hz) was applied in the case of FES using VFTs. The authors concluded that novel FES systems capable of delivering VFTs during gait can result in an enhanced drop-foot correction as opposed to the traditional FES systems that deliver CFTs. The results also suggested that the delivery timing of FES during gait was critical and that it is also required further investigation. Concerning doses, Smith et al. (2003) have demonstrated a dose-response relationship between FES and brain activation in sensory and motor regions contralateral to the stimulation and it is also suggested that more intensive training may be beneficial (Bosch et al., 2014). In relation to the electrical stimulation orthotic substitute devices, Bosch et al. (2014) pointed out that the limited total intervention time, small sample size, and lack of focus on gait training raise questions about the optimal interventions and training intensity needed to obtain beneficial effects.

There is evidence supporting the use of electrical stimulation for therapeutic training and as a replacement or alternative to an AFO (Bosch et al., 2014). These positive effects may include changes in gait characteristics and body function variables (Kafri et al., 2015). The

therapeutic effect of FES on balance did not demonstrate any clear patterns. In addition, consistent findings indicate that when FES is used as an alternative to an assistive device it has no superior therapeutic effects than AFO (Kafri et al., 2015; Bosch et al., 2014).

In terms of patient characteristics, it is not clear which individuals will benefit most from NMES, and what baseline characteristics predict better therapeutic outcomes (Kafri et al., 2015). Merletti et al. (1979) suggested that the time since lesion, spasticity, and intensity of treatment are important predictors of positive therapeutic results.

The use of FES combined with different walking strategies has been shown to result in improvements in hemiplegic gait (Belda-Lois et al., 2011). These methods include cycling, leg movements in a side lying position, treadmill training, electromechanical gait training and so on. FES has also been applied in conjunction with mirror therapy indicating good results in gait ability, muscle strength and balance in hemiplegic stroke survivors (Ji et al., 2014; Lee et al., 2016). Preliminary studies have been carried out in relation to a multi-level NMES paradigm with a virtual reality-based adaptive response technology for post-stroke balance rehabilitation (Dutta et al., 2014). NMES can also be used along or as a part of a neuro-robot (Moreno et al., 2011), yet this evidence is still limited. Future research topics have been suggested in relation to the combination of FES and Lokomat-assisted robotic rehabilitation therapy that might lead to further mobility improvements (Jezernik et al., 2003).

Locomotion results from intricate dynamic interactions between a central programme and feedback mechanisms (Belda-Lois et al., 2011). While functional changes in mobility performance may be attributed to the direct effects on muscle structure and function, two other central mechanisms may be involved namely the direct effects of electrical stimulation on brain plasticity and the enhanced motor learning resulting from repetitive practice (Kafri et al., 2015). Findings indicate that NMES in conjunction with voluntary muscle contractions is superior to individual NMES in terms of cortical excitability and plasticity (Kafri et al., 2015).

HEMIPLEGIC SHOULDER

The hemiplegic shoulder constitutes a challenge in the rehabilitation of stroke patients. Shoulder subluxation may affect the upper limb treatment process. It can also lead to additional complications, such as pain and delays in functional recovery (Vafadar et al., 2015; Gu et al., 2016).

Various meta-analyses and reviews have been published on this subject in recent years. Sheffler et al. (2007) argued that the use of NMES appears to reduce shoulder subluxation along with the pain-free ROM, as well as facilitate motor recovery, especially in the case of acute stroke patients. However, additional studies are required in order to clarify the clinical efficacy thereof. The conclusions formulated by Vafadar et al. (2015) and Gu et al. (2016) indicated similar findings. Both authors pointed out that NMES could be used in order to prevent or reduce shoulder subluxation early after a stroke, yet findings did not support their efficacy for pain reduction, improvement in arm strength, movement, functional activity, and quality of life following a stroke.

The NMES protocols applied to the hemiplegic shoulder are very disparate and clinical trials do not fully describe them in the majority of cases, thus making it difficult for a

comparison to be made between the studies. The stimulated musculature, which includes the supraspinatus and the posterior deltoid, is the only variable that most studies have in common, whereas the rest of the stimulation parameters vary from one author to another. The frequency ranges mainly between 10 and 30 Hz, the most frequent pulse duration is 300 μ s, although on many occasions these values are not defined by the author. The waveform as along with the intensity parameters also fail to be indicated in the vast majority of studies. Furthermore, ramping up/down periods and duty cycles are very different in the defined protocols, which is also the case for the treatment doses.

More extensive and accurate studies should be carried out in order to reach conclusive results about the long-term effects of NMES and their relation to the post-stroke time period (Vafadar et al., 2015; Gu et al., 2016).

DYSPHAGIA

Dysphagia or swallowing problems are common and they occur in 45-65% of patients following an acute stroke (Daniels et al., 1998; Lee et al., 2014). Complications of dysphagia include aspiration leading to chest infections and pneumonia, malnutrition, inability to rehabilitate, prolonged length of hospitalisation, and an increased risk of death (Geeganage et al., 2012). Different interventions are applied in order to accelerate the recovery of the swallowing function. NMES for the treatment of dysphagia is a relatively new therapeutic method (Poorjavad et al., 2014; Tan et al., 2013; Park et al., 2012). This new approach has generated a lot of interest across a vast amount of publications in recent years, yet the available evidence is limited (Chen et al., 2016; Poorjavad et al., 2014).

A recent meta-analysis (Tan et al., 2013) evaluated the effect of NMES versus traditional therapy (TT) in patients with dysphagia of different aetiology. In the subgroup analysis of stroke patients, the effectiveness of NMES and TT was comparable, patients with dysphagia may benefit to a greater extent from NMES when combined with TT. Following the same approach, the latest meta-analysis (Chen et al., 2016) concluded that swallowing treatment by means of NMES seems to be more effective than the interventions without it in the case of post-stroke dysphagia in the short term, and the authors remarked that the evidence is insufficient in order to indicate whether NMES alone is superior to swallowing therapy.

Related to the NMES protocol, after reviewing various clinical trials, the device predominantly used is the Vitalstim® equipment (Chattanooga Group, Hixson, TN, USA, device approved by the FDA for the treatment of dysphagia), including biphasic pulses, a frequency of 80 Hz and a pulse duration of 700 μ s. The stimulation intensity is adjusted according to the motor or sensory threshold, the usual dosage of treatment is 60 min per day for 5 days per week, with the duration of the study fluctuating between 5 days and 6 weeks. On the same subject, a review (Poorjavad et al., 2014) related to the dysphagic patients following a stroke, analysed the intensity of stimulation (sensory or motor level) and the electrode placement at the level of the neck in more detail. The authors pointed out that these two parameters of the protocol could have important effects on the outcomes and they had not been extensively considered by previously published reviews (Steele et al., 2007; Clark et al., 2009; Tan et al., 2013). Generally, NMES on the swallowing muscles has been applied with two general purposes, namely to cause muscle contractions in which case the intensity of the

electrical current is increased until the muscle contraction occurs, and to stimulate the sensory pathways in which case the sensory threshold is usually identified as the lowest current level at which the patient feels a tingling sensation in the neck skin. Electrode placement usually involves the suprahyoid or infrahyoid muscles. According to this review, the majority of the studies reported some positive treatment outcomes in the case of the electrical stimulation to the neck musculature in relation to the swallowing function of stroke patients, especially when the intensity of the stimulus was adjusted at the sensory level or when the motor electrical stimulation was applied to the infrahyoid muscles when swallowing. Nevertheless, additional studies ought to be carried out in relation to the physiologic and neurologic effects of these therapeutic methods, which would help to design specific and individual treatment protocols. Rofes et al. (2013) compared the efficacy and safety of 10 days of treatment with NMES being recorded at sensory and motor intensities in chronic post-stroke patients. The authors concluded that NMES improved the swallow response and safety of swallow both at sensory and motor intensities, and the efficacy of swallow following the motor treatment. A more significant effect was observed when the stimulus was applied at motor intensities.

The mechanisms underlying the efficacy of electrical stimulation in the treatment of post-stroke dysphagia are not well-known. Gallas et al. (2010) reported that the plasticity of the sensory swallowing cortex is presumed to be based on the long-term reorganisation of the human cortex driven by the sensory stimulation. Rofes et al. (2013) and Chen et al. (2016) suggested that both peripheral and central modifications could be responsible for the effect of the therapy, whereas Sun et al. (2013) mentioned that NMES could improve the hyolaryngeal elevation, restore motor function of weak muscles, combat disuse atrophy, enhance sensory awareness and facilitate muscle contractions.

URINARY INCONTINENCE

Urinary incontinence (UI) following a stroke affects more than one-third of acute stroke patients (Liu et al., 2016), namely between 40% and 60% of patients who are hospitalised, 25% of patients upon hospital discharge, and 15% of patients one year after stroke (Thomas et al., 2011). NMES has also been proposed as a treatment tool for the patients with post-stroke urinary symptoms (Skeil et al., 2001; Cooperberg et al., 2005), although there is a paucity of existing research about it. The rehabilitation of patients with post-stroke UI is still a relatively new topic, and its occurrence has not been identified as a problem by therapists, even in developed countries (Guo et al., 2014).

Guo et al. (2014) investigated the therapeutic effect of TENS on post-stroke UI. The treatment group received therapy by means of TENS for 30 minutes once a day over the course of 60 days, at a pulse duration of 70 μ s and a frequency of 75 Hz. In terms of the electrode placement, the positive electrode was placed on the second lumbar spinous process, whereas the two negative electrodes were located on the inside of the middle and the lower third of the junction between the posterior superior iliac spine and the ischia node. The daily micturition, nocturia, urgent urination, and urge UI in the treatment group improved significantly compared to the control group. Later, the same authors published another related article (Liu et al., 2016), comparing two protocols in which TENS was applied at different frequencies, namely 20 Hz and 75 Hz, and pulse durations of 150 μ s, for 30 min once per day

over the course of 90 days. This time, two positive electrodes were used, which were placed in the region of the second sacral level on opposite sides of the vertebral column, whereas the negative electrodes were placed in the same manner as the previously published article. The stroke patients treated with a frequency of 20 Hz had superior Overactive Bladder Symptom Scores, Barthel Index totals, urodynamic values and voiding diary parameters. Despite obtaining promising results, the authors recommend that more studies involving larger sample sizes and longer follow-up periods ought to be conducted in order to confirm the long-term effects of electrical stimulation.

VISUO-SPATIAL NEGLECT

Visuo-spatial neglect is a neurological disorder that often follows a stroke, especially after sustaining damage to the right hemisphere of the brain. It refers to the defective ability of patients to explore the side of the space contralateral to the brain lesion and to report stimuli presented in that portion of the space (Vallar et al., 1998). Different types of interventions are suggested. Electrical stimulation is investigated in some studies, although the use thereof is not very widespread and well-known.

There are few clinical trials in the case of this specific population. Many of these investigations include samples with other neurological disorders that also sustain brain lesions. Vallar et al. (1995) reported that TENS applied to the muscles on the left side of the neck led to a temporary improvement in 93% of neglect patients due to different neurological disorders. Left-hand stimulation had a comparable effect. Schröder et al. (2008) recommend TENS as a possible visual neglect therapy based on the results of their research. They compared different treatments, namely exploration training alone and in combination with TENS or optokinetic stimulation (OKS). In the case of TENS group, the stimulation frequency was set at 100 Hz with a pulse duration of 100 μ s (as in the study of Vallar et al., 1995). The positive electrode was placed over the left trapezius muscle halfway between the head and the shoulder and the negative one was attached about 40 mm below, on the upper left shoulder. The intensity was individually determined by increasing it gradually until a pleasant tingling in the neck was reported. Each patient received 20 therapy sessions each lasting between 25 and 40 min over a period of 4 weeks. The results of this RCT indicated that the groups receiving TENS or OKS showed significant improvements in their reading and writing abilities, although the improvement in neglect test scores disappeared during the follow-up period in TENS group. Pérennou et al. (2001) demonstrated the manner in which it is possible to modulate the postural instability in neglect patients by means of an appropriate somatosensory manipulation, namely the use of TENS. The authors concluded that TENS stimulation applied at the neck level could be a convenient tool for inducing postural neuromodulation in a clinical context. However, the duration of the TENS efficiency with reference to the postural stability lasted for a short period of time. In this regard, further investigation is needed to determine whether repeated applications of TENS eventually associated with some active retraining techniques will facilitate the functional restoration of neglect patients after a stroke.

The specific mechanisms underlying this intervention are complex and unclear, but the latter findings suggest that TENS may elicit a proprioceptive input through large diameter

afferents (Vallar et al., 1995). Vallar et al. (1995) suggested that the improvement could be attributed to a resulted from a non-specific activation of the right hemisphere or a directional effect on the egocentric coordinates of the extrapersonal space. The activation of the neural systems involved in the organization of the egocentric representation has also been suggested by Schröder et al. (2008) and Pizzamiglio et al. (1996). Lomber and Payne (1996) regarded the unilateral neglect as the result of an imbalance in the activity of the two hemispheres at the cortical or subcortical level. In this manner, TENS might serve to reduce this imbalance by increasing the activity of the right hemisphere.

CONCLUSION

Advances in neuroscience and the development of new technologies will possibly give rise to new applications of NMES for upper limb recovery after a stroke. Future NMES protocols should take into account various aspects including bilateral activity, repetitive movement, maximum subject attentiveness, and tasks that motivate goal-driven motor skills. These variables are suspected to promote motor relearning and to enhance the recovery of upper limb in stroke patients. NMES could be a complement to rehabilitation programmes along with other emerging therapies. Further research is needed to establish whether the parameters, treatment doses and patient characteristics are crucial for the effectiveness thereof. Such research should also elucidate the specific mechanism of action associated to the different stimulation modalities in order to select the most suitable patients and to optimise individual treatment protocols to achieve larger treatment effects.

Further research is also required in order to investigate the neurobiological principles that underlie any functional changes in lower limb and gait, to elicit the subpopulation of stroke patients that will most benefit therefrom and to evaluate their effectiveness in line with other emerging strategies. These studies would also be useful in order to optimise the protocols applied. Lower limb and gait programmes should include training principles such as goal-directed, task-specific training, intensive in terms of the number of repetitions, challenging, and motivating, in an attempt to facilitate motor learning on the basis of neural plasticity. In addition, further technological advances are expected to increase the availability and usability of gait FES systems, while in turn significantly contributing to gait and quality of life of post-stroke patients. These new systems should be associated with cost effectiveness studies in order to assess its viability in clinical practice.

In relation to the hemiplegic shoulder, It would be interesting to carry out RCTs with the aim of comparing different treatment protocols in order to determine which parameters are the most effective, as well as to consider whether the treatment programme could be different depending on the patient characteristics. An accurate assessment of the shoulder to be treated and a more homogeneous selection of the sample could provide more information about the effectiveness of NMES on the hemiplegic shoulder and establish protocols in accordance with the treatment purpose.

With reference to the future lines of research in relation to post-stroke dysphagia, there is a need to carry out future controlled studies with larger group of patients and a long-term follow-up period. New findings could thus be used in order to optimise the electrical stimulation parameters, to investigate the role of lesion sites and time from stroke, and to

study the neurophysiologic measurements of cortical excitability with the purpose of confirming the action mechanism of the therapy.

Future research is needed to obtain more concluding results in the case of novel strategies associated to urinary incontinence and visuo-spatial neglect in order to establish effective treatments.

It is therefore important to possess extensive knowledge about the effects of NMES in these different areas and to further investigate their maintenance during the follow-up period, in order to guide clinical decisions and the bioengineering community in the development of appropriate technologies. New advances in nanotechnology, e-textiles and telerehabilitation will play an interesting role in future stroke rehabilitation. A combination of different rehabilitation strategies seems to be more effective as opposed to the individual implementation thereof. Future research would make it possible to analyse the impact of rehabilitation on brain plasticity, in order to adapt treatment resources to meet the needs of each patient and to subsequently optimise the recovery process. More rigorous clinical and economic evidence is needed to incentivise practitioners and healthcare decision-makers to implement these NMES treatments. In addition, a multidisciplinary interactive effort between engineering, neuroscience and medicine is required to progress in the field of neurorehabilitation.

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Chapter 94

MULTIMODAL MONITORING IN DECOMPRESSIVE CRANIECTOMY FOR TRAUMATIC BRAIN INJURY AND STROKE

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ABSTRACT

The popularity of multimodal monitoring (MMM) in guiding treatment has grown over the past decade, despite slight evidence to support its use. Clarity is lacking about the indications for MMM, when it should be instituted, and how the collected data should be interpreted. Also in question are which MMM physiologic variables are most clinically pertinent and whether a monitoring-targeted approach improves outcomes. Although the contemporary concept of individualizing treatment based on patient-specific physiologic variables has gained increasing favor, few clinical data support this approach. Relying on therapy guided by monitoring of intracranial pressure and cerebral perfusion pressure is proving to be insufficient in addressing the pathophysiology of critical neurologic conditions. Understanding the limitations and challenges of MMM is crucial to interpretation of the data. Moreover, integration of the continuously monitored data and defining the phenotype of brain injury by a cumulative analysis are essential. The integration of MMM into clinical practice is evolving. Recently, several computational systems allowing the integration of the data into electronic medical records have been developed. The concept of individualizing physiologic parameter thresholds and regimen needs further prospective clinical validation.

Keywords: multimodal monitoring, traumatic brain injury, stroke, individualized treatment, data integration, limitations of multimodal monitoring, indications of multimodal monitoring, multimodal monitoring and clinical outcomes

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INTRODUCTION

Care of patients undergoing decompressive craniectomy for traumatic brain injury or stroke lies in preventing and mitigating secondary brain injury through timely intervention, thus improving outcomes. To achieve the best outcome, appropriate physiological variables such as ICP, CPP, PRx, blood flow velocity, PbtO₂, lactate/pyruvate ratio are monitored and any significant changes are managed accordingly. Appropriate decision making is hindered when insufficient data are obtained from the neurological exam and neuroimaging. These static measures do not provide sufficient information on the dynamic pathophysiology that drives secondary brain injury. Data acquisition using several tools such as the neurological exam, neuroimaging, and systemic and neurophysiological parameters, i.e., multimodal monitoring (MMM), can guide individualized care. This approach is promising in detection of early changes before irreversible damage ensues. In this chapter, we discuss the different parameters for MMM, their integration in decision making, and the challenges and limitations they present to those using them.

INTRACRANIAL PRESSURE AND CEREBRAL PERFUSION PRESSURE

Some fundamental concepts undergird our understanding of the dynamic relationship between intracranial pressure (ICP) and cerebral perfusion pressure (CPP). The Monro-Kellie doctrine, for example, provides the conceptual basis for treating elevated ICP. Human intracranial volume is constant and is a sum of the volumes of cerebrospinal fluid, brain, blood, and mass lesion. An increase in the volume of any one of these components beyond the compliance of the brain inevitably leads to elevated ICP. Lundberg [1] introduced the concept of slow waves of ICP and interpretation of pathologic waveforms. Later, Langfitt et al. [2] and Lofgren and Zwetnow [3] introduced the pressure–volume curve and the term *compensatory reserve*.

ICP Wave Forms

The ICP wave form comprises three prominent components: pulse wave form, respiratory wave form, and slow waves. These components overlap in the time domain but can be separated in the frequency domain. The pulse wave form has several components of which the fundamental component correlates with heart rate and represents the mean ICP. The amplitude of this component is valuable in extracting various indices. ICP wave has pulsatile quality at two different frequencies, one synchronous with the arterial pulse and the other synchronous with the respiration. The respiratory waveform is related to the frequency of the respiratory cycle (8–20 cycles per minute). Slow waves commonly are not as explicit as in the original Lundberg thesis [1]. The cerebrospinal fluid pulse waveform is depicted in Figure 1. The three classically described patterns of ICP fluctuation reported by Lundberg are known as A, B, and C waves. Figures 2 and 3 depict Lundberg type A and B waveforms. C waveforms are not thought to have pathophysiological importance.

Pressure Volume Compensatory Reserve

The brain has a compensatory mechanism when there is an increase in one of the compartments with volume changes in the others. This compensatory mechanism defines the compliance of the brain. When these compensatory mechanisms fail, a small volume change can cause a significant ICP increase or even herniation. Compliance is vital since a patient with poor compliance is at a greater risk than one with good compliance, even if that patient's ICP is normal. The compensatory reserve can be plotted by defining the relationship between the mean ICP and changes in volume of the intracerebral space, known as the pressure–volume curve. This index is called the RAP [4], i.e., the correlation coefficient [R] between the amplitude [A] and the mean pressure [P]. An interpretation of RAP as an index delineates where the cerebrospinal system “working” point rests on the pressure–volume curve. An RAP coefficient nearing 0 signifies a lack of synchronization between changes in the amplitude of the fundamental component and the mean ICP. This represents a good pressure–volume compensatory reserve at low ICP, where a change in volume produces no or very minor change of the pressure. On the other hand, when RAP increases to +1, the amplitude of the fundamental component varies proportionally to the ICP. The curve of intracranial space shifts toward the right, toward the steep part of the pressure–volume curve. In this phase, the compensatory reserve is low and thus any additional increase in the volume will lead to a sharp rise in ICP.

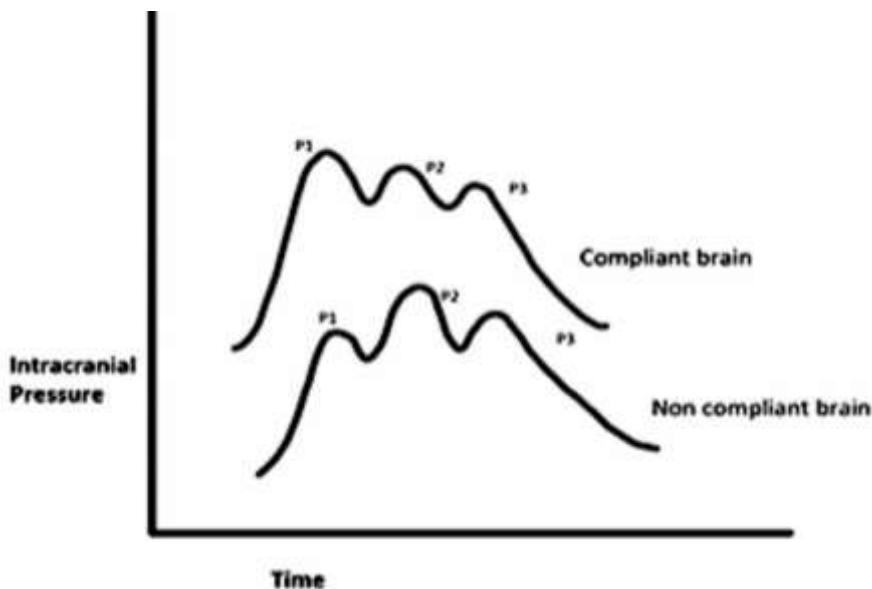


Figure 1. Sketch indicating the three components of ICP monitoring tracing, (1): percussion wave (P1), tidal wave (P2), and dicrotic wave (P3). The percussion wave correlates with the arterial pulse transmitted through the choroid plexus into the cerebrospinal fluid, and via a column of fluid into the external ventricular drain (EVD) transducer. The tidal wave represents the cerebral compliance. The amplitude of P2 generally increases as compliance decreases, and when the P2 exceeds the P1 waveform, a marked decrease in cerebral compliance exists. The dicrotic wave correlates with the closure of the aortic valve, which makes the trough prior to P3 the equivalent of the dicrotic notch. (Courtesy of N Badjatia, University of Maryland).

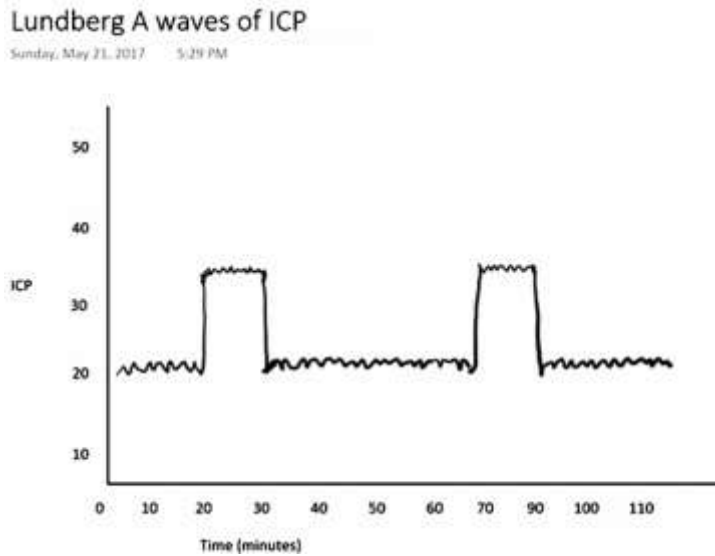


Figure 2. Sketch indicates 2 classic Lundberg type A waves. These are steep rises in ICP to a plateau of 40 mm Hg or higher and are sustained for 5 to 20 minutes before falling rapidly. They represent a critical reduction in intracranial compliance. Plateau waves are associated with working cerebrovascular reactivity and low cerebrospinal compensatory reserve (Courtesy of N Badjatia, University of Maryland).

METHODS OF ICP MONITORING

ICP monitoring and treatment are best performed through a multimodal approach that encompasses clinical examination and imaging modalities. The intraparenchymal and intraventricular catheters are two preferred techniques in widespread use for ICP monitoring. Both are invasive devices and carry minimal risk of infection and bleeding when placed by skilled operators [5]. The intraventricular catheter is considered a gold standard and represents a global ICP assessment in comparison to the intraparenchymal monitor. In addition, it aids in cerebrospinal fluid diversion into the external ventricular drainage (EVD) and hence is an important resuscitation tool, especially in cases of acute hydrocephalus. It may have additional benefits with lower rates of refractory ICP and improved outcomes over intraparenchymal monitors [6]. The intraventricular catheter can also assist in delivering intraventricular drugs such as a thrombolytic for clearance of intraventricular hemorrhage [7]. Intraparenchymal monitors are relatively easier to place than intraventricular catheters. A major downside of intraparenchymal monitors is that they can give an inaccurate reading in cases where there is focal pathology leading to compartmentalization of ICP due to cerebral hemispheric and tentorial boundaries [8].

A change in baseline ICP readings, called a zero drift, associated with microtransducer systems in the intraparenchymal probes can result in measurement error over a certain period. In addition, parenchymal devices have limitations regarding recalibration *in vivo* and durability [9]. A recent meta-analysis showed both types of monitors yield similar average values, but interval values could differ [10]. Choice of which method to use is decided on an individualized basis and factors such as the etiology of the brain injury, coagulopathy,

accuracy, reliability, complication rates, and the cost associated with the devices [11]. With transition to electronic charting, a new paradigm of automated data collection allows for more accurate recording of data and holds intriguing future applications. Manual recording of ICP is not an accurate representation of the true pattern of unstable ICP in patients with traumatic brain injury (TBI) [12]. Automated continuous dynamic monitoring of mean arterial pressure (MAP) and ICP allows accurate prediction of episodes of intracranial hypertension and long-term outcomes in the neurosurgical intensive care unit [13].

Noninvasive Modalities for ICP Monitoring

Noninvasive techniques such as transcranial Doppler (TCD) and optic nerve sheath diameter (ONSD) have been utilized for ICP monitoring, especially when an invasive modality is contraindicated, not clearly indicated, or not available. TCDs can assess ICP through several methods including pulsatility index, noninvasive assessment of CPP, and several mathematical models. Depending on the method, there is considerable intra- and inter-group variability in reporting accuracy. Using the pulsatility index, Bellner and colleagues demonstrated encouraging results [14]. They cited a robust relationship between the pulsatility index and ICP with a correlation coefficient of $R = 0.94$ ($P < 0.05$). However, other studies have reported a weaker correlation between the pulsatility index and ICP [15, 16]. Studies have reported a similar variance in accuracy of monitoring ICP when using CPP estimation with TCDs. Czosnyka et al. estimated CPP using flow velocity and arterial blood pressure and reported a correlation coefficient of $R = 0.73$ between noninvasive CPP measurements and CPP measured through a parenchymal monitor. The estimation error was less than 15 mm Hg in up to 84% of cases [17]. TCD, however, is insensitive in cases where the elevation of ICP is not vasogenic in origin [18]. There can be technical difficulties in cases where an adequate acoustic window for artery insonation is not present. Precision of the tool is also dependent on operator skills. Despite of these limitations, TCD is good screening tool for ICP. ONSD can be measured using an ultrasound, and a 4.5- to 5.5-mm ONSD roughly correlates to an ICP of 20 mm Hg [19, 20]. ONSD has been shown to be much more predictive of ICP than computed tomography (CT) features in severe TBI patients [21]. The robust data on ONSD reported by Rajajee et al. [19] suggested an ONSD >4.8 mm correlated to an ICP >20 mm Hg with a sensitivity of 96% and a specificity of 94%. Other studies have reported a lower sensitivity and specificity [22]. Although ONSD is a quick tool to assess ICP, it provides only a snapshot of information. ONSD varies with individual factors such as age and underlying pathology, making it difficult to create a threshold for ICP treatment. This tool has good utility as a screening tool and in places lacking resources for continuous ICP monitoring. Another noninvasive approach of ICP measurement is an automated Neurological Pupil index obtained using a pupilometer. All of the above techniques have limitations, such as lacking the capability of continuous detection of changes, and need further clinical validation. Still, they serve as good screening tools and provide easy means of performing repeated assessments.

Indications for ICP Monitoring

The Brain Trauma Foundation (BTF) “Guidelines for the Management of Severe Traumatic Brain Injury” advocates monitoring ICP in all salvageable patients with a severe TBI (Glasgow Coma Score [GCS] 3–8 after resuscitation) and an abnormal CT scan (revealing hematomas, contusions, swelling, herniation, or compressed basal cisterns). This position was supported by Level IIB evidence [23]. ICP monitoring should be established in patients with severe TBI with a normal CT scan if two or more of the following features are noted at admission—age over 40 years, unilateral or bilateral motor posturing, or systolic blood pressure <90 mm Hg. The reason is that the incidence of intracranial hypertension was almost 60% when at least two of these features were present [24].

The consensus conference of recognized experts on ICP monitoring in TBI has made several additional recommendations [25]:

- Monitoring the ICP of comatose TBI patients with a normal initial head CT is generally not recommended. Repeat CT is recommended to rule out any new abnormalities after an early initial CT. Patients with new or worsening CT findings should be monitored.
- ICP monitoring is recommended following a secondary decompressive craniectomy to assess its effectiveness in controlling ICPs and to guide further therapy.
- ICP monitoring after evacuation of an acute supratentorial intracranial hematoma should be considered for salvageable patients with features associated with an increased risk of elevated ICPs. The following are the pre-operative and intra-operative clinical findings warranting ICP monitoring in supratentorial lesions: a GCS motor score ≤ 5 (the risk was felt to be even higher if ≤ 4); pupillary abnormalities (anisocoria or bilateral mydriasis); prolonged/severe hypoxia and/or hypotension; compressed or obliterated basal cisterns; midline shift exceeds 5 mm; midline shift exceeds thickness of an extra-axial clot; additional extra-axial hematomas, parenchymal injuries (such as contusions) or swelling and evidence of intraoperative brain swelling.
- Comatose patients with an initial CT scan demonstrating diffuse injury with signs of brain swelling (e.g., compressed/absent basal cisterns) should have ICP monitoring.
- Comatose patients with traumatic brain contusions, in whom the interruption of sedation to check the neurological status is considered dangerous or when the clinical examination is not completely reliable, should be subjected to ICP monitoring.
- Primary decompressive craniectomy (i.e., absence of bone flap) is not a sufficient reason for not monitoring the ICP in the postoperative period if any indications for ICP monitoring are present.

ICP Monitoring in Traumatic Brain Injury and Outcomes

CPP optimization and control of raised ICP are fundamental in addressing the primary and secondary pathophysiologic processes in TBI. ICP is an important marker of injury

severity in TBI [26]. Increased ICP is associated with mortality and poor outcomes [26-30]. Even transient episodes of ICP elevation are associated with poor outcomes [30]. In addition, the pattern and duration of rise and the total dose of ICP are consequential in defining outcomes [27, 31, 32]. ICP treatment responsiveness or refractoriness also affects outcomes [26, 33].

As indicated by the BTF guidelines, moderate quality data exist from multiple observational studies demonstrating benefits of treatment guided by ICP monitoring in the form of decreased in-hospital and 2-week post-injury mortality [34-37]. Aggressive ICP-guided care has also been shown to be more cost effective than routine care [38]. The consensus summary statement of the International Multidisciplinary Consensus Conference on Multimodality Monitoring in Neurocritical Care mandates ICP monitoring when other intracranial monitors are used, to provide a framework for optimal interpretation [11]. Retrospective studies [39, 40] emphasize the importance of monitoring ICP post-decompressive craniectomy, having found better outcomes in the group where the management strategy was based on ICP monitoring.

The Benchmark Evidence from South American Trials: Treatment of Intracranial Pressure (BEST TRIP) showed no superiority of treatment based on ICP monitoring versus intervention based on protocolized neurological exam and imaging [41]. ICP-guided treatment was, nonetheless, more efficient in treating ICPs than the other approach. BEST TRIP, however, was not a trial of ICP monitoring or the efficacy of ICP monitoring, but rather a study of two methods of severe TBI management. The results were informative on the need for further study on selection of patients for ICP monitoring, determination of patient-specific ICP thresholds, and development of treatment protocols and strategies [42].

The recent RESCUEicp trial (Randomised Evaluation of Surgery with Craniectomy for Uncontrollable Elevation of ICP) [43] addressed some of the key limitations of the DECRA (Decompressive Craniectomy) trial evaluating the role of decompressive craniectomy for management of refractory ICP [44]. The RESCUEicp trial compared last-tier secondary decompressive craniectomy with continued medical management in patients with a higher ICP threshold (25 mm Hg for 1 to 12 hours) despite maximal medical treatment except for barbiturates. The study showed benefit in the surgical arm in terms of lower mortality, but a higher number of patients in a vegetative state or with severe disability. The RESCUEicp trial was effective in reducing refractory ICPs, but provided no benefits to the pathophysiology of primary neurologic insult.

Despite current recommendations and clinical practice, Level 1 evidence is lacking on whether therapy guided by ICP monitoring makes a difference in clinical outcome. There is no randomized control trial (RCT) addressing treatment threshold in TBI. The data on target ICP number are derived from several observational studies [23, 24, 45]. An increase in ICP above 22 mm Hg is associated with unfavorable outcomes [46], and is currently the treatment threshold in the BTF guidelines as a Level 2B recommendation [23].

Although CPP can be derived by monitoring ICP, the adequacy of cerebral perfusion cannot be reliably determined by an ICP number. Secondary brain injury can occur even with normal ICP and CPP numbers [47]. Bouzart et al. [48] made this point emphatically by showing that MMM incorporating brain tissue partial pressure of oxygen monitoring in addition to ICP can identify cerebral hypoperfusion more reliably than ICP monitoring alone.

The ICP value is dependent on the interplay of several factors, including cerebral compliance, perfusion, oxygenation, and cellular functioning, that vary in different

pathophysiological states. In addition, the intensity of the treatment is driven by patient's response/resistance to treatment, which predicts the severity of the insult in several disease states but does not alter the underlying pathology. Hence, targeting isolated CPP/ICP addresses the supply to brain without considering the underlying cerebral metabolism.

Treatment decisions regarding ICP tend to focus on individualizing thresholds based on patient characteristics, cerebral physiology, and risk–benefit considerations. The approach of treating ICP thresholds is not pathophysiology-specific and is a generalized measure. Hence, the approach to ICP treatment is more phenomenological than mechanistic. It does not allow individualizing therapy and is more reactive than proactive. This underlines the significance of MMM to intervene appropriately on pathophysiological states and individualizing care rather than merely treating an ICP number.

CEREBRAL BLOOD FLOW, CEREBRAL PERFUSION PRESSURE, AND ISCHEMIA

Hypotension significantly impacts outcomes after TBI [49, 50]. Adequate perfusion to the brain is essential in preventing secondary brain injury driven by hypoxia, ischemia, and cerebral metabolism.

Cerebral Blood Flow

Cerebral blood flow (CBF) is regulated and affected by several varied factors such as arterial blood pressure, intracranial pressure, venous outflow, blood viscosity, partial pressure of carbon dioxide or oxygen in arterial blood (PaCO₂ and PaO₂, respectively), collateral flow, vasoreactivity, and the status of cerebral autoregulation. Alterations in the PaCO₂ exert a profound influence on CBF, with an approximately 4% change in CBF for every 1-mm Hg change in PaCO₂ in either direction. This arteriolar response is mediated by a local effect of H⁺ ions or pH fluctuations in the extracellular fluid surrounding vessels in the brain [51]. The PaO₂ in the normal physiological range does not affect CBF, but when PaO₂ falls below 50 mm Hg, CBF increases dramatically. CBF can be measured both directly and indirectly; however, direct measurement is challenging. For this reason, surrogate variables have been used to monitor and treat patients with TBI and stroke. CBF can be directly measured using a thermal diffusion probe (Hemedex) inserted into brain parenchyma. The probe measures the thermal gradient between the distal thermistor and the proximal temperature sensor, providing a regional CBF measured in milliliters/100 grams brain tissue/minute. Normal CBF in humans varies widely depending on tissue demands and age, but averages around 50 mL/100g/minute. Irreversible neuronal damage occurs in a time-dependent manner when CBF is below 10–15 mL/100 g/minute, whereas reversible neuronal dysfunction has been noted at a CBF between 15 and 20 mL/100 g/minute.

TCD is a well-studied, noninvasive, and easily reproducible tool for estimating CBF. TCDs provide an indirect estimate of CBF based on flow velocity through a cerebral vessel. A linear relationship exists between CBF and middle cerebral artery flow velocity if the cross-sectional area and angle of insonation are constant. The Lindegaard ratio [52], the ratio

of the middle cerebral artery velocity to the internal carotid velocity, can signify if the increase in the middle cerebral artery (MCA) velocity is related to vessel narrowing.

The strategy of hyperventilation is often used in the acute setting of TBI and elevated ICP. Excessive hyperventilation in TBI can result in ischemia and is associated with poor outcomes [53, 54]. An end tidal CO₂-guided hyperventilation strategy and assessment of CBF can therefore be crucial in these patients. In addition, CBF assessment is also crucial in patients requiring sedation and paralysis with mechanical ventilation. TCDs can help estimate CBF at bedside under these circumstances.

Although positron emission tomography (PET) is the accepted gold standard for assessment of CBF and brain metabolism, its prohibitive cost and limited accessibility in many trauma centers have restricted the widespread clinical use of PET in the acute setting. As an alternative, xenon-enhanced CT has been validated as a method for measuring CBF and guiding management in TBI [55-57]. Xenon-enhanced CT provides a measurement of the CBF. Ischemia within 6–12 hours following trauma as detected by xenon CT studies in TBI patients has been associated with adverse long-term outcome [58].

Arterial spin labeling (ASL) is a magnetic resonance imaging (MRI) technique that enables the measurement of brain perfusion noninvasively at the tissue level. ASL works on the principle of magnetically labeled blood obviating the need of contrast. ASL is a sensitive modality to detect structural brain damage and outcome in mild TBI [59]. Application of ASL depicts mismatch perfusion in acute stroke and helps identify tissue at risk in chronic cerebrovascular disease. ASL can be used to estimate the cerebrovascular reserve and future risk of cerebrovascular events [60].

Cerebral Perfusion Pressure

CPP is defined as the difference between the MAP and the ICP. This represents the pressure gradient driving CBF and hence oxygen and metabolite delivery. The current BTF guidelines on CPP recommend a threshold of 60–70 mm Hg in TBI patients [23]. Different strategies to optimize CPP in TBI patients have been studied [61, 62]. Patients who have an impaired autoregulatory status will have increased cerebral blood volume and increased risk of secondary brain injury with higher CPPs due to elevated ICPs and hyperemia [63]. Loss of autoregulation puts the patient at risk of secondary brain injury via ischemia with hypotensive episodes. On the other hand, patients with intact autoregulatory status would be better served with higher CPPs. Depletion of the cerebrovascular reserve and impaired autoregulation leads to direct dependence of CBF on CPP; hence the CBF may still be suboptimal despite the CPP being within normal range [64]. The adequacy of the CBF and CPP is also dependent on the cerebral metabolic oxygen consumption. Despite decreased CBF, there would be no ischemia if the cerebral metabolic rate of oxygen (CMRO₂) is decreased (e.g., in sedation). To the contrary, when CBF reduction is greater than the CMRO₂, ischemia and eventually secondary brain injury ensue. The ultimate goal for CPP depends on the autoregulatory status of the patient. Optimal CPP, i.e., the range of CPP at which the autoregulatory status is intact, can be calculated using the pressure reactivity index (PRx). Several studies have assessed the relationship between optimal CPP, neurologic outcomes (65-68), and physiologic outcomes [64] in TBI. Jaeger et al. demonstrated that CPP above optimal has no benefit on brain tissue oxygenation after TBI [64]. Large deviations from CPP above optimal on either side are

associated with poor outcomes [68]. CPP augmentation puts patients at risk for systemic complications (e.g., ARDS), for demand ischemia in other organs, and for poor outcomes [62, 69]. Targeting optimal CPP can be much more effective than having a range of CPP thresholds in preventing secondary brain injury and complications. However, there still is not enough evidence to incorporate a single number for CPP into clinical practice [70].

Cerebral Autoregulation

Under normal physiological conditions, cerebral autoregulation is a complex process that is a byproduct of the interplay between myogenic, neurogenic, and metabolic mechanisms. Cerebral autoregulation is a protective hemodynamic mechanism developed to protect the brain from changes in CPP causing either hypoperfusion or hyperperfusion. Autoregulation normally maintains MAP between ~ 50 and 150 mm Hg. Normal CBF in humans varies widely depending on tissue demands and age but averages around 50 mL/100 g brain tissue/minute. Acute brain injury, however, often leads to dysfunctional autoregulatory mechanisms lasting up to 2 weeks [71]. The assessment of pressure autoregulation can be classified as static or dynamic [72]. Static assessment illustrates changes in the CBF measures due to fluctuations in MAP. Dynamic assessment takes into account the time factor when looking at changes in CBF in response to MAP. There are two fundamental approaches to optimizing therapy and treating patients with TBI. One approach is the Lund concept [73, 74] that is based on managing increased ICP and regulating cerebral volume. The other is an aggressive CPP management [61] to prevent ischemia and secondary brain injury. Howells et al. [75] suggested that the most appropriate of the two approaches for individual patients may depend on the state of cerebrovascular PRx. PRx is a dynamic assessment of autoregulation.

Pressure Reactivity Index

Cerebrovascular pressure reactivity is the ability of vascular smooth muscle to respond to changes in transmural pressure. As described by Czosnyka and colleagues, PRx is a computer-aided method of calculating a correlation coefficient between 40 past consecutive 5-second averages of ICP and arterial blood pressure (ABP); computations were repeated with a moving window every 5 seconds [76]. Current standards for computing PRx in monitoring are modified to a moving correlation coefficient between 30 values of consecutive 10-second ABP and ICP averages. If pressure reactivity is intact, a rise in MAP will lead within 5–15 seconds to vasoconstriction with reduction in cerebral blood volume, and thus will lower ICP. If there is dysregulation in pressure reactivity, cerebral blood volume will increase passively, and ICP will rise. When there is a reduction in MAP, the opposite takes place. This technology has been used to develop individualized thresholds for ICP and CPP above optimal. Information on pressure reactivity can potentially guide treatment decisions for intracranial hypertension based on the cerebral physiology.

A positive PRx suggests a positive association of ABP and ICP, reflecting a passive non-reactive vascular bed (Figure 4). A negative value of PRx reflects a normally reactive vascular bed, representing an inverse association between ABP and ICP. Patients can have impaired autoregulation even with mild TBI, making them more susceptible to hypotension.

Impaired cerebral autoregulation will lead to sudden increases in MAP being easily transmitted into the microcirculation and can contribute to secondary brain injury in the form of ischemia/ infarction, hyperemia, cerebral edema, or cerebral hypoxia [63]. Furthermore, cerebral autoregulation determines the response to the treatment administered to a patient with severe TBI, including hyperosmolar therapy and vasopressors. A positive PRx correlates significantly with high ICP, low admission GCS score, and poor outcome at 6 months after injury [76]. PRx correlates well with cerebral autoregulation flow velocity in TCD ultrasonography, static rate of autoregulation based on TCD.

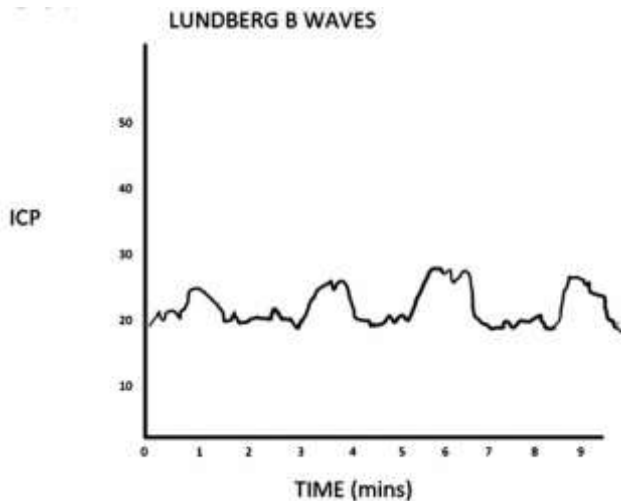


Figure 3. Sketch indicates Lundberg type B waves. Lundberg type B waves occur with a frequency of 0.5 to 2 Hz and are rhythmic oscillations to 20–30 mm Hg above the baseline but without a sustained period of intracranial hypertension (Figure 3) (Courtesy of N Badjatia, University of Maryland).

The PRx has been validated against a PET-derived static rate of autoregulation based on PET and oxygen extraction fraction [77]. Patient-specific ICP thresholds calculated based on PRx and the total ICP dose were shown to be more robust predictors of 6-month clinical status and mortality than the conventional thresholds of 20 and 25 mm Hg [78]. In patients after decompressive craniectomy, PRx might not be as useful unless clearly positive, because of the considerably high compliance of intracranial cavity after skull opening. Limitations of the PRx lie in measuring accurate MAPs and CPP calculations, which can have significant implications for treatment based on them [79]. Despite optimal CPP/ICP numbers, cerebral hypoxia can still occur [80, 81]. Studies assessing the interplay between cerebral microdialysis, PET imaging, and brain tissue oxygen tension (PbtO₂) monitoring have demonstrated that despite normal ICP/ CPP parameters, cerebral hypoxia and secondary brain injury can occur [47, 82–84]. A CPP/ICP-targeted approach might therefore be suboptimal in TBI patients. For this reason, there has been a recent emphasis on characterizing the cerebral physiology and metabolism and on individualizing treatment based on the patient's clinical profile. This approach could offer an edge in guiding therapy and preventing potential deleterious side effects of the treatment strategies (Figure 5).

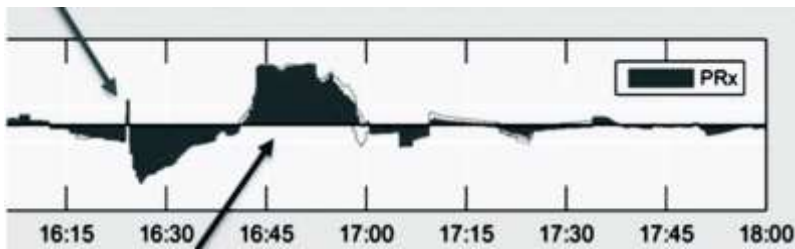


Figure 4. Sketch indicating a positive correlation coefficient (pointed out by the long arrow at 16:45) between changes in mean ICP and MAP (PRx) which signifies poor pressure-reactivity of cerebral arterioles (Courtesy of N Badjatia, University of Maryland).

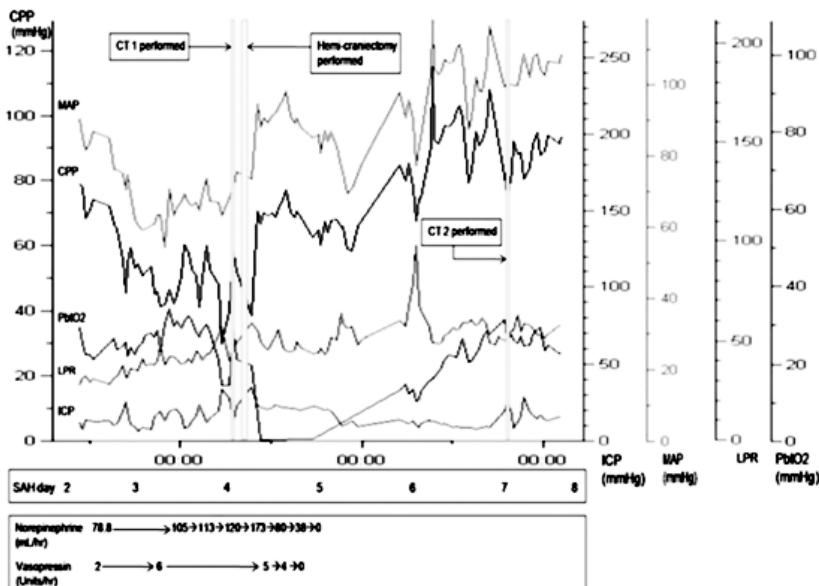
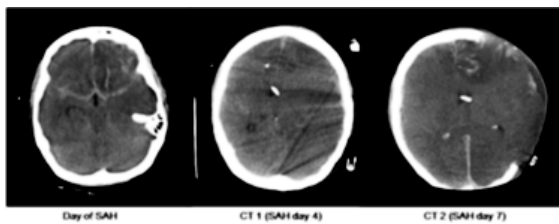


Figure 5. Example of MMM changes preceding hemicraniectomy in a subarachnoid hemorrhage patient (reference: Stuart et al. *Neurocrit Care* (2011) 15:146–150). Intracranial pressure (ICP), cerebral perfusion pressure (CPP), mean arterial pressure (MAP), lactate: pyruvate ratio (LPR), and brain tissue oxygen tension (PbtO₂) trends for post-bleed days (SAH days) 2–8. Infusion rates of norepinephrine and vasopressin are also displayed, as well as timing of head CT's. Before craniectomy, note the decrease in CPP and MAP despite increasing rates of norepinephrine and vasopressin, as well as the early rise in LPR >40, decrease in CPP <40 mm Hg, and late decrease in PbtO₂ <25 mm Hg. In the hours immediately preceding hemicraniectomy, the LPR was actually greater than the CPP. After craniectomy, ICP was maintained <25 mm Hg and CPP >60 mm Hg, and all pressors were weaned to off within hours. The PbtO₂ probe malfunctioned immediately post-surgery (Courtesy of N Badjatia, University of Maryland).

Brain Oxygenation

While PET is considered the gold standard in monitoring brain tissue oxygenation, other modalities include jugular venous oxygen saturation (SjVO₂), brain tissue oxygenation (PbtO₂), oxygen reactivity index (OR_x), near-infrared spectroscopy (NIRS), and MR spectroscopy. Imaging modalities provide snapshots of information versus dynamic information obtained through continuous modalities. BTF guidelines recommend placement of a brain oxygenation monitoring device when hyperventilation strategies are employed. More recent MMM guidelines recommend placement of a PbtO₂ device in patients at risk for ischemia [11, 23].

SjVO₂

Oxygenation of cerebral venous outflow has been utilized as a neuromonitoring modality for several decades [85, 86]. SjVO₂ provides an estimate of global cerebral oxygenation status and adequacy of the CBF. The jugular bulb, the dilated portion of the jugular vein just below the base of the skull, is the preferred site for blood sampling. Even though the jugular bulb drains bilateral hemispheres, usually the right internal jugular vein is the dominant side of drainage, making it the preferred side for insertion in diffuse disease processes. In cases of a focal brain injury, it is controversial whether the catheter insertion site should be ipsilateral to the brain injury side or on the dominant side, if a difference exists. There is significant disparity between two jugular vein oxygen saturation measurements [87]. Needle puncture, retrograde central venous catheter into the jugular vein, or insertion of a fiber optic catheter all allow for a continuous SjVO₂ monitoring. Skull x-ray can be used to confirm placement. On a lateral neck radiograph, the catheter tip should be at the level of and just medial to the mastoid process.

Important formulas related to the physiology of brain oxygenation and utilization are as follows:

$$DO_2 = CBF \times CaO_2$$

$$CMRO_2 = CBF (CaO_2 - CjvO_2)$$

$$A_jvDO_2 = CMRO_2 \div CBF$$

$$CBF = \frac{\Delta P}{4 \pi r^4 \eta L}$$

$$\text{Arterial Oxygen content} = (1.34 \times Hgb \times PaO_2) + (0.0031 \times PaO_2)$$

where DO₂ = delivery of oxygen, CBF = cerebral blood flow, CaO₂ = arterial oxygen content, CMRO₂ = cerebral metabolic rate of oxygen, CjVO₂ = jugular venous oxygen content, Hgb = hemoglobin, SjVO₂ = jugular venous oxygen saturation, A_{jv}DO₂ = difference in oxygen content between arterial and jugular venous blood, PaO₂ = arterial oxygen saturation, r = radius, P = pressure, Z = viscosity, and L = length. CBF is governed by Poiseuille's law.

Normal SjVO₂ ranges from 55% to 75%. SjVO₂ is an indirect assessment of cerebral oxygen consumption. When demand exceeds supply, the brain extracts greater oxygen, resulting in decreased jugular bulb oxygen saturation. If CBF decreases, eventually the compensatory mechanism of increased oxygen extraction in response to decreased CBF will fail and result in anaerobic metabolism with lactate production. When cerebral oxygen supply exceeds demand, SjVO₂ is elevated.

SjVO₂ monitoring has numerous clinical implications. For one, it promptly diagnoses ischemia resulting from either cerebral or systemic causes [88-92]. SjVO₂ < 55% suggests ischemia or increased metabolism and oxygen extraction, whereas SjVO₂ > 75% indicates hyperemia and decreased metabolic demand. Low values are associated with poor outcomes especially in the setting of no improvement with CPP augmentation [93]. SjVO₂ can guide treatment decisions regarding fluid strategy, optimization of CPP and oxygenation [11, 23, 89], and detecting arteriovenous fistulas [94]. ICP management, including utility of barbiturates [95] for suppressing cerebral metabolism, is also guided by SjVO₂ monitoring [11, 23].

Complications associated with SjVO₂ include carotid artery puncture, pneumothorax, injury of cranial nerves IX and X, infection, and thrombosis. Appropriate placement of the catheter in the jugular bulb is crucial to avoid extracranial contamination and inaccurate information. The interpretation of SjVO₂ and CBF can be challenging by itself, since the assumption is that the CMRO₂ remains constant. In addition, SjVO₂ can be insensitive to focal ischemia. High SjVO₂ values can be falsely reassuring because they may, in fact, be associated with pathologic arteriovenous shunting, brain death, or cerebral infarction.

PbtO₂

PbtO₂ has become the gold standard for monitoring brain oxygenation. The limitations of CPP/ICP monitoring to detect ischemia/hypoxia are avoided by PbtO₂ monitoring [48]. It assists in better understanding the underlying pathophysiology of secondary brain injury. PbtO₂ is affected by oxygen demand and supply including perfusion and oxygenation [96-99]. However, several factors such as relative proportion of arterial and venous supply in the region of interest (shunting) and diffusion barriers (interstitial/cellular edema) [100], cerebral metabolism (fever, seizures, shivering), and systemic factors like PaCO₂, PaO₂ [101-104] affect PbtO₂. Hence, PbtO₂ is more of a marker of cellular and mitochondrial functioning. Rosenthal et al. showed that a PbtO₂ number is the result of CBF and the arteriovenous oxygen tension difference rather than being a surrogate of cerebral metabolism and oxygen delivery [105]. That PbtO₂ can be low despite a normal CBF suggests microcirculatory collapse and edema [100]. This can be clinically significant when assessing MMM data to guide treatment.

Normal PbtO₂ ranges from 25 to 35 mm Hg [106-108], with the most recent MMM guidelines suggesting 20 mm Hg as the treatment threshold [11]. Ischemic thresholds have ranged between 10 and 19 mm Hg in the literature [109-111]. PbtO₂ < 5 mm is associated with cell death [112-114]. The clinical significance of the PbtO₂ number varies based on the probe placement [109]. When the probe is placed in the normal tissue and autoregulation is intact, PbtO₂ has a good correlation with CPP. In penumbral tissue, the correlation with CPP can be inconsistent. When placed in dead tissue, there is no correlation with CBF/ CPP.

PbtO₂ has various methods of measurement. Biomedical engineering includes optical luminescent and polarographic methods. The polarographic method is much more common, and has been used in the development of the LICOX® (Integra Neurosciences, Plainsboro, NJ) brain tissue monitoring system, commonly used in clinical practice. The NEUROVENT®-PTO (Raumedic Co., Mills River, NC) multimodal catheter uses optical luminescence methodology. The LICOX monitor is inserted into the brain parenchyma like a fiber optic ICP monitor, generally 3.5 cm below the dura in the frontal white matter. It measures the partial pressure of oxygen around the catheter insertion site, which measures

about 17 mm³ of brain tissue around the distal end. In TBI, the monitor is usually inserted into the normal brain tissue in the frontal lobe ipsilateral to the severely injured brain. In cases of diffuse injury, the monitor is usually inserted into the non-dominant hemisphere.

PbtO₂ monitoring has numerous clinical implications. In TBI, several observational studies have reported the effect of brain hypoxia on outcome. The presence of cerebral hypoxia has been associated with unfavorable outcomes at 6 months and increased mortality [47, 115-117]. Increasing duration of hypoxia [111] and early hypoxia within the first 72 hours after brain injury [118] have been shown to predict mortality. The duration and burden of brain tissue hypoxia have also been associated with worse outcome at 30 days [47, 84]. PbtO₂ has been established as an independent predictor of outcomes. Low PbtO₂ is not specific for simple perfusion failure. Factors such as PCO₂, PaO₂, and hypermetabolic states can all affect PbtO₂. PbtO₂ number signifies a balance between supply, which is dependent on perfusion, and demand, which is the magnitude of oxygenation and extraction. Although a PbtO₂-guided intervention strategy in TBI has been studied, the data on outcome benefits are still unclear [119-122]. The BTF guidelines recommend PbtO₂ monitoring in patients with severe TBI to complement ICP/ CPP monitoring [23]. PbtO₂ monitoring can be used to individualize thresholds for CPP in conjunction with ICP monitoring [123].

ORx

The oxygen reactivity index (ORx) is a surrogate of pressure autoregulation and has some prognostic value. The potential to improve PbtO₂ using vasopressors by increasing the MAPs and CPP is dependent on the status of cerebral autoregulation. When the autoregulation is intact, increasing the CPP usually has a negligible effect on the PbtO₂, and compensatory vasoconstriction leads to the CBF and oxygen delivery remaining constant. In the event of the autoregulation being impaired or when the CPP is outside the autoregulatory range, either too low or too high, CBF and brain PbtO₂ correlate directly with CPP augmentation, leading to elevation in brain PbtO₂. The ORx is calculated as the moving linear (Pearson) correlation coefficient between PbtO₂ and CPP and ranges from +1 to -1. When autoregulation is intact, ORx is near 0 or negative. When autoregulation is impaired, PbtO₂ passively follows CPP, and ORx is positive. Recent studies, however, have refuted the relationships between PRx and ORx and between ORx and PbtO₂ [124].

ORx has the advantage of being one of the most effective real time bedside monitoring tools available for detecting focal ischemia. It provides focal monitoring for tissue at risk of secondary brain injury, and it is a relatively safe modality. But its use does have limitations. Because it is a focal invasive monitor, ORx can miss distant changes in the tissues. It can have sensitivity drift requiring recalibration. There is a “run in” period immediately after the probe insertion, when the readings are inaccurate. “Oxygen challenge” should be performed daily post insertion to verify accurate functioning of the probe. Placing the probe into the precise location is challenging, and the probe can give an inaccurate assessment if it is inserted into the lesion instead of viable tissue. Critical thresholds have been ascertained through patient outcomes in small studies rather than pathophysiologic evidence of ischemic damage at the cellular level.

NIRS

Near-infrared spectroscopy (NIRS) in the TBI population is best applied in detection of mass lesions, midline shift, and cerebral edema because of high sensitivity [125]. NIRS is

most suitable for use in the outpatient setting for early detection, resuscitation, and quick transfer of patients to well-equipped tertiary centers. Use of NIRS to detect intracranial pathology in the inpatient setting is not widespread owing to readily available CT scan and imaging modalities.

Although the application of NIRS in monitoring ICP has been studied, the exact relationship between information obtained from NIRS and severity of ICP is not quite clear [126, 127]. In addition, there is no convincing evidence that NIRS can be reliably combined with any other invasive monitoring modality (ICP, tissue oxygen tension, jugular bulb saturation), and NIRS cannot be used in place of these modalities when they are indicated. The correlation of PbtO₂, SjvO₂, and microdialysis with the information obtained from NIRS data is inconclusive. The use of NIRS in TBI is therefore not widespread, owing to concerns about extracranial tissue and lesions interfering with the modality. Current devices monitor change in brain oxygenation. Another limitation of NIRS is that the thresholds for ischemia/hypoxia are not well determined.

Systemic Oxygenation

Systemic venous oxygenation is not a surrogate of SjVO₂. The brain receives approximately 15% to 25% of the total cardiac output and has a high extraction capacity and a low oxygenation reserve compared to other organs. Brain PbtO₂ correlates with systemic oxygenation and PaO₂. For example, acute lung disease will lead to a lower brain PbtO₂, independent of cerebral and systemic injuries [128]. The ratio of the change in PbtO₂ to the change in PaO₂ is described as the tissue oxygen response. The brain responds to hyperoxia in the form of cerebral vasoconstriction [129, 130], thus reducing CBF, a brain protective mechanism related to oxygen toxicity. The tissue oxygen response is another marker of cerebral autoregulation, like PRx and ORx.

Cerebral Metabolism and Microdialysis

Cerebral metabolism, which is significantly altered in acute brain injury, is roughly divided into three phases after brain injury: hypermetabolism lasting hours to days, hypometabolism lasting days to weeks, and transition into normal metabolism lasting weeks to months (this third phase is less well established) [131]. MMM guidelines recommend cerebral microdialysis as a clinical tool in patients with or at risk of cerebral ischemia, hypoxia, energy failure, and glucose deprivation [11].

Cerebral microdialysis requires that a specialized catheter with a semipermeable dialysis membrane be placed into the brain parenchyma through a burr hole and secured by a cranial bolt. The catheter is perfused with a cerebrospinal fluid-like solution, and sampling of brain extracellular fluid is conducted periodically to assess concentrations of lactate and pyruvate, ischemic metabolites, glucose, glycerol, markers of cell membrane damage, glutamate, and excitatory amino acid. The best of these biomarkers is the lactate/pyruvate (L/P) ratio, which implies a shift from aerobic to anaerobic metabolism and has prognostic significance [11]. L/P ratios >25 have been suggested as critical thresholds for metabolic crisis [132, 133]. Metabolic crisis can occur despite normal ICPs [132] and is associated with poor outcomes.

Energy crisis has been found to occur, characterized by normal PbtO₂ and elevated lactate with normal/low pyruvate levels, without evidence of ischemia or defect in oxygen delivery related to mitochondrial dysfunction [82]. In addition, L/P ratio has been associated with several causes such as low CPP [134] and thresholds of hemoglobin [135]. Hence, the L/P ratio can have significant clinical implications when using strategies to maximize perfusion.

Several diverse types of brain tissue hypoxias and neuromonitoring profiles have been described in the literature ([136, 137] Table 1). PET studies have found a variable relationship between cerebral metabolic rate for oxygen, L/P ratio, and oxygen extraction fraction depending on the location of the probe, timing of monitoring, and thresholds for ischemia [82, 138]. The type of hypoxia defines the relationship between L/P ratio and oxygen extraction fraction [139]. Interpretation of dialysate components might be confounded by drugs, nonconvulsive status epilepticus, and enzyme deficiencies (140). A reduced brain/serum glucose ratio [141] signifies metabolic crisis and can help differentiate other confounders causing high L/P ratio. In defining critical causes of cerebral perfusion, Bouzat et al. [48] defined a better role for PbtO₂ and cerebral microdialysis than ICP monitoring alone. The safety profile of cerebral microdialysis used to determine L/P ratio is similar to any other probe in MMM. Because it provides a focal measurement, its limitations lie in the metabolic collection assessment based on the location between injured and preserved brain. Cerebral microdialysis is a cumbersome technique and metabolite collection occurs over time. Hence, the data interpretation and usage can be delayed. Moreover, the benefits of targeting L/P ratio thresholds in terms of outcomes are unclear. The ideal location for regional monitors of MMM has not been identified. Conventional practice is placement in brain parenchyma at the greatest risk for secondary injury. For focal injury, such as ischemic stroke or traumatic intracerebral hemorrhage or contusion, that location is directly adjacent to the damaged area. In global injury, the non-dominant frontal cortex is used.

Table 1. Neuromonitoring profiles and treatment approaches, by category of brain tissue hypoxia

Category of hypoxia	Neuromonitoring profile	Treatment approach
Ischemic	↓CBF, ↓CPP ~ ICP ↓ PbtO ₂ ↑LPR	CPP augmentation with fluid and/or vasopressors Improve oxygen delivery
Hypermetabolic (Demand supply mismatch)	↑CBF, ↑CPP ~ ICP ↑PbtO ₂ ↑LPR	Evaluate and treat for causes such as fever, shivering, ictal-interictal EEG patterns

Electrophysiology

Electroencephalography (EEG) is a noninvasive, continuous modality utilized in detecting seizures, ischemic changes, new structural lesions, and recovery of altered mental status following TBI or stroke. EEG provides information on global and regional cerebral functioning. The rationale for long-term EEG monitoring lies in detecting subclinical seizures as an etiology for altered mental status or coma. In addition, it is hoped that early detection

and suppression of seizures, either convulsive or nonconvulsive, would translate into improved outcomes [11, 142, 143]. Incidence of nonconvulsive seizures in TBI has been reported to be up to 22% (144), whereas the incidence of early seizures in acute stroke ranges from 3% to 33%, with 50% to 78% of the seizures occurring within the first 24 hours [145]. Occurrence of seizures within 24 hours of stroke is associated with higher 30-day mortality [145]. Vespa et al. [146] demonstrated a high incidence of nonconvulsive status epilepticus and a relationship between acute posttraumatic nonconvulsive status epilepticus and disproportionate long-term hippocampal atrophy.

Fluctuations in CBF have been linked to changes on the EEG [147], (Figures 5 and 6) and different cellular responses. When CBF drops to 25–35 mL/100 g/minute, the EEG first loses faster frequencies due to anaerobic metabolism and glutamate release at a cellular level. As CBF continues to plummet to about 17–18 mL/100 g/minute, slower frequencies increase, with generation of lactic acidosis and depletion of ATP at the cellular stage. This represents an ischemic threshold (reversible). As CBF declines to 10–12 mL/100 g/minute and lower, sodium-potassium pump failure, an increase in cellular water content, and eventually cell death take place. Cell death is marked by severe suppression on continuous EEG. Diffusion-weighted imaging (DWI) is capable of detecting ischemic changes in stroke at CBF 35–40 mL/100 g/minute within 30 minutes [148]. In comparison, continuous EEG can detect ischemic changes within seconds and provide a window of opportunity to intervene, especially in the case of a mismatch between DWI-identified lesions and clinical assessment.



Figure 6. A short strip EEG belonging to a subject following TBI manifesting radiographic evidence of left internal carotid artery (ICA) vasospasm due to post traumatic subarachnoid hemorrhage. There is evidence of slowing of electrical waves over the left hemisphere (Leads with odd numbers) (Courtesy of N Badjatia, University of Maryland).

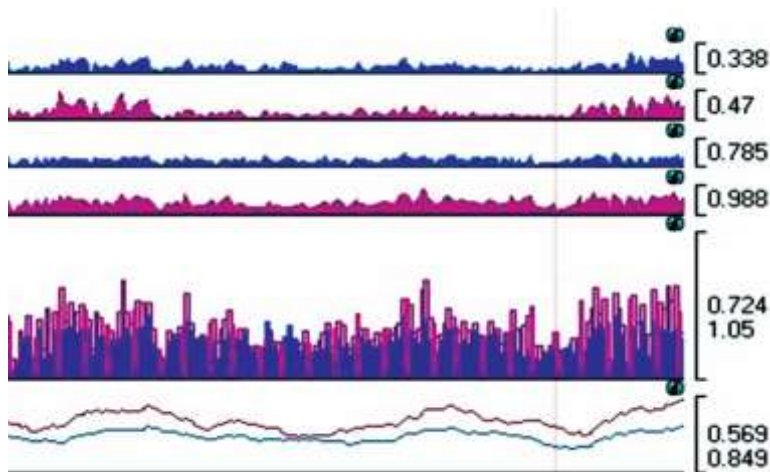


Figure 7. Quantitative analysis of EEG belonging to a subject following TBI manifesting radiographic evidence of left internal carotid artery (ICA) vasospasm due to post traumatic subarachnoid hemorrhage. There is evidence of difference in the Alpha Delta Ratio (ADR) between the left (0.569) and right hemisphere (0.872) (Courtesy of N Badjatia, University of Maryland).

EEG can assist in guiding treatment with either tissue plasminogen activator [149, 150] or augmentation of blood pressure or CBF after an acute ischemic stroke [151]. Quantitative EEG limits subjectivity in EEG interpretation, obviating the need for trained personnel to interpret EEG in the intensive care unit. With Fourier transformation, EEG can be quantified in terms of its amplitude, power, frequency, and rhythmicity, and numerical values, ratios, or percentages can be obtained. When applying quantitative EEG in acute ischemic stroke, the hemispheric relative delta percentage and the overall mean frequency correlate well with CBF [152]. Increase or decrease in the power of slower and faster frequencies varies with the changes in the cerebral metabolism [153]. Continuous EEG can be used to monitor neuropathological mechanisms and to detect markers of secondary brain injury [154] (Figures 6 and 7). These markers are manifested as periods of isolated or continuous depression in the form of high amplitude delta activity induced by spreading cortical depolarization, a feature of all acute brain injury. There are some data on the role of EEG in prognostication in patients with acute brain injury (TBI/stroke). Loss of reactivity [155] and lack of sleep-wake cycles [156] may portend poor prognosis (and indicate possible brainstem involvement). On the other hand, the presence of faster frequencies within 24 hours is consistent with a good outcome [157].

Glucose and Nutrition

Early sympathetic surge related to brain injury can cause dysfunction of glucose control. Hyperglycemia not only is a marker of disease severity in TBI but also can worsen secondary injury. In a study conducted on 59 patients with moderate and severe brain injuries, serum glucose levels >200 mg/dL in the first 24 hours after hospital admission were associated with worse neurologic outcome at hospital discharge, 3 months, and 1 year following trauma [158]. Cerebral microdialysis holds great promise in helping to understand the

pathophysiology of metabolic demand after brain injury and thereby providing an individualized goal. Studies using microdialysis have shown that tight glucose control can exacerbate brain metabolic crisis [159]. In ischemic brain injury, hyperglycemia has been associated with worse outcomes [160]. The Stroke Hyperglycemia Insulin Network Effort trial [161] will be informative in terms of the range of blood glucose to target in acute ischemic stroke.

Temperature and Inflammation

Target temperature management in TBI has been studied under two hypotheses. The first concerns mitigating secondary brain damage caused by ischemia/reperfusion injury and inflammation. The second is based on the role of hypothermia in ICP management. The National Acute Brain Injury Study: Hypothermia (NABIS:H) found no evidence of improved outcomes (Glasgow Outcome Scale at 6 months) in patients who were maintained on a hypothermia regimen for 48 hours after injury [162]. In a later report, NABIS:H II did not show any beneficial effect on morbidity or mortality for patients with severe brain injury [163]. However, subanalyses of RCTs and some animal data suggest a benefit of early, preoperative hypothermia in patients with focal brain lesion [164]. An international multicenter RCT (HOPES trial) is underway assessing the role of hypothermia in acute subdural hemorrhage. More recently, a multicenter RCT to examine the efficacy and safety of long-term mild hypothermia in severe TBI is planned in China (the LTH-1 trial).

The Eurotherm3235 [165] trial was a multicenter RCT assessing hypothermia as a second-tier treatment strategy along with standard medical management versus only medical management with an ICP threshold of 20 mm Hg. The study was stopped prematurely owing to safety concerns, but the results suggest that outcomes were worse with hypothermia than with standard care alone. Several questions have been raised about the trial concerning the timing of hypothermia in general practice and the treatment threshold of ICP used in the study. In a case control study, maintaining normothermia reduced the number of ICP spikes greater than 25 mm Hg, leading the authors to recommend fever avoidance in TBI patients [166]. Currently, the role of hypothermia in TBI is unclear.

Acute Stroke and the Role of Multimodal Monitoring

The use of MMM in acute stroke is far less common than in TBI. Still, the indications for MMM in acute ischemic/hemorrhagic stroke are like those discussed in the section on TBI above. The International Multidisciplinary Consensus Conference on Multimodality Monitoring has made recommendations regarding which non-TBI patients are suitable for ICP and CPP monitoring [167]:

1. Stroke patients with poor neurological exam either before or after decompressive hemicraniectomy.
2. Patients at risk for elevated intracranial pressure/hydrocephalus
3. Patients with supratentorial large hemispheric lesions or large infratentorial lesions

4. Patients with intraventricular hemorrhage with a GCS <8 (poor neurological exam) should be considered for an EVD and ICP and CPP monitoring
5. Patients with deep ischemic/hemorrhagic stroke (at risk for obstructive hydrocephalus) with imaging and clinical exam mismatch should be considered for MMM

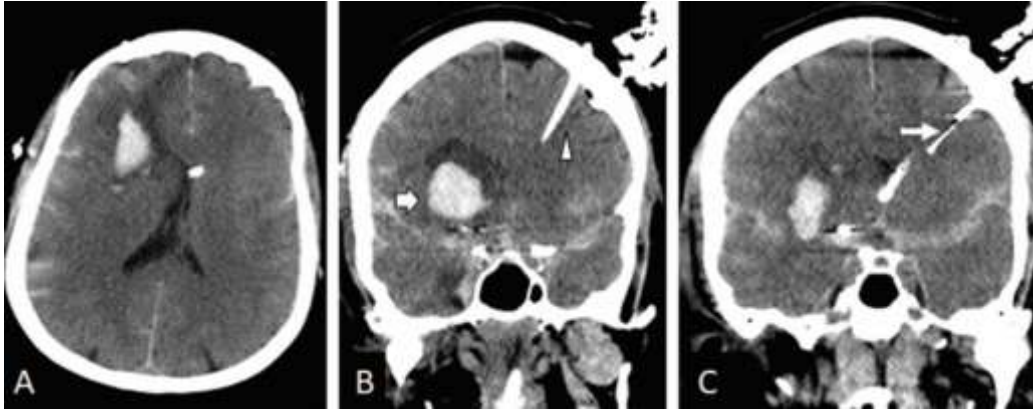


Figure 8. CT images of the head showing diffuse subarachnoid hemorrhage and a frontal parenchymal hematoma with surrounding edema and midline shift. The tip of the EVD is seen in the left lateral ventricle on the axial imaging (Plate A). Coronal sections (Plates B and C) indicate the tip of the Licox probe placed into the normal brain parenchyma (Courtesy of N Badjatia, University of Maryland).

Location of Monitoring

There is no consensus on the ideal location of the MMM probe and the EVD site in the stroke population. The location of the probe should be in the area at highest risk of secondary injury. Thus, in stroke patients the best location is in the penumbra or perihematoma area [168] (Figure 8). However, in cases of severe unilateral damage or if the patient has already undergone a hemicraniectomy, the monitors are inserted into the contralateral areas near the side of lesion. There is also a concern for compartmentalization of elevated ICP in focal brain lesions at a deep or infratentorial location. Because of these limitations, the ICP numbers can be inaccurate and cerebral herniation can ensue without a rise in ICP [169].

ICP and CPP Monitoring in Stroke

When elevated ICP is a concern in management of stroke due to either intraventricular hemorrhage or cerebral edema, EVD is considered the gold standard for monitoring ICP [170, 171]. If intraparenchymal probes are used, the ICP sensor placed contralateral to the ischemic hemisphere can lead to an inaccurate estimation of ICP. Few, if any, studies have shown benefit with therapy guided by ICP monitoring in stroke care. American Heart Association/American Stroke Association guidelines recommend that patients with a GCS ≤ 8 , those with clinical evidence of transtentorial herniation, and those with significant intraventricular hemorrhage or hydrocephalus might be considered for ICP monitoring and

treatment. A CPP of 50 to 70 mm Hg has been recommended depending on the state of cerebral autoregulation [170, 171]. Regardless of the underlying physiologic differences in ICP elevation, maintaining proper CPP before initiating ICP-lowering treatments is of utmost importance. ICP monitoring can give us an understanding of autoregulation and cerebral physiology in stroke. Optimal CPP targets can be delineated for individual patients, and thus assist in pathophysiological goal-directed therapy. A single center using MMM consisting of ICP probe, PbtO₂ monitor, and cerebral microdialysis in intracerebral hemorrhage showed the risk of brain tissue hypoxia was higher with CPP <80 mm Hg than CPP >100 mm Hg [172]. In addition, survivors in the study had consistently lower elevations in their L/P ratio and PRx, showing intact cerebral autoregulation compared to those with fatal outcomes. One retrospective observational study utilizing ICP monitoring-based treatment in patients with stroke after decompressive hemicraniectomy showed effective treatment of ICP, but no studies have addressed differences in outcome with such treatment [173].

Cerebral Blood Flow in Stroke

TCDs can be used as a modality for estimating CBF. TCD is less accurate than CT angiography and MR angiography for steno-occlusive disease, with a sensitivity and specificity of 55% to 90% and 90% to 95%, respectively [170]. TCD usage is limited by the skills of the interpreter and the patient's vascular anatomy. Moreover, TCD is not applicable for posterior circulation strokes.

Cerebral Microdialysis in Stroke

In a small prospective clinical study of patients with malignant space-occupying infarction, monitoring showed a substantial increase in concentrations of glutamate and lactate and in the L/P ratio [174]. Overall, the data on cerebral microdialysis are limited in stroke. Data on PbtO₂ and SjvO₂ are obtained from the TBI studies, and very few studies have looked into their roles in the stroke population [175].

Monitoring of Cerebral Edema in Stroke

Malignant MCA stroke represents 2% to 8% of all hospitalized ischemic stroke cases, and MCA territory ischemic stroke accounts for 10% to 15% of patients hospitalized for stroke [176]. Clinical examination, cortical signs, NIH Stroke Scale, early pupillary abnormalities, and level of consciousness have been associated with malignant MCA and cerebral edema [176]. Neurological deterioration can occur within 72 to 96 hours, and sometimes up to day 10. In the cerebellar strokes, compression of brainstem structures and development of obstructive hydrocephalus will manifest as decline in consciousness with cranial nerve abnormalities [176]. CT is a readily available, safe first-line test that can be used to assess cerebral edema. Several CT findings have been shown to predict development of malignant edema and poor prognosis: hypodensity involving >50% MCA territory on CT within 6 hours, involvement of a third or more of the MCA, and a midline shift ≥ 5 mm within

2 days [176]. MRI is not as widely available and thus might not be suitable for every patient. The data on MRI to predict development of malignant edema suggest a superiority of MRI over CT. Two studies evaluating MRI within 6 hours found similar cutoffs for DWI volume, around 80 mL, to be highly predictive, with specificities of 50% to 95%, but sensitivities of 50% to 85%. A DWI volume cutoff of >145 mL for an MRI done in 14 hours achieved a 100% sensitivity and 94% specificity [176, 177].

Limitations of Multimodal Monitoring

MMM is limited in the following ways:

1. It is resource intensive and cumbersome.
2. It requires expertise in interpreting the complex data sets.
3. There is a lack of evidence on improved outcomes using this treatment strategy.
4. Data can be inaccurate depending on the limitations of the modalities and the factors affecting them.

Beyond the obvious challenges of expense, large number of human and technological resources needed, and requirement for expertise in interpreting data, some other problems are less obvious. Chief among these are acquisition, integration, and synchronization of MMM data. Electronic medical records have eased recordkeeping through automated data collection, and they provide more reliable documentation. Unfortunately, many monitors do not interface with electronic medical records. As a result, the data are being captured continuously, but recorded only intermittently. Clearly, further development of continuous data integration into electronic medical records with a user-friendly interface is required. This will help clinicians simultaneously visualize all of the data at any given time, interpret it, and then apply that knowledge to patient care.

CONCLUSION

The integration of MMM in the field of neurointensive care holds great promise for early recognition of worsening brain injury and improving the efficacy of treatment strategies. It is a powerful monitoring tool in cases where the neurologic exam is limited. MMM allows for individualized care rather than a general approach based on the collected continuous data. It helps us detect changes in cerebral physiologic and metabolic states. In addition, the effect of treatment strategy can be observed in real time and the approach can be tailored accordingly. Interpreting and acting on the different physiologic variables can be challenging and have several limitations. The accuracy of several probes and devices can vary based on several factors. Because clinical data on improved outcomes based on treatment guided by MMM are limited, further studies analyzing the application of MMM in improving patient outcomes are required.

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Chapter 95

DECOMPRESSIVE CRANIECTOMY IN ISCHEMIC STROKE

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ABSTRACT

Few stroke syndromes have as high a risk of mortality or severe disability as does the “malignant” middle cerebral artery infarction seen in the setting of large artery occlusions. Malignant stroke syndromes are relatively rare; however, when present they are associated with a close to 80% mortality rate. The management of these patients is controversial and presents a major challenge for the neurologists, neurosurgeons, and neurointensivists caring for them. Many studies have assessed the benefits and risks of decompressive craniotomy versus optimal medical management, with the goal of improving not only survival but also survival with good functional outcomes. The conclusions of these studies remain mixed, and practice patterns vary widely. We review the landmark trials of decompressive surgery in ischemic stroke as well as the pooled analyses and those addressing quality of life to improve our understanding of this complicated syndrome in which the optimal management strategy may vary depending on a patient’s values and preferences.

Keywords: malignant stroke, decompressive craniotomy, large artery occlusion, quality of life, functional outcomes

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INTRODUCTION

Stroke is defined as the sudden onset of neurologic disability caused by neuronal death due to blockage or severely reduced blood flow or hemorrhage within the brain parenchyma. Currently, stroke is the world's second leading cause of mortality and third for disability [1]. In the United States, stroke is now the fifth leading cause of death, is one of the top causes of long-term disability, and costs the nation nearly 33 billion dollars annually [1]. Larger territories of parenchymal damage typically equate to higher levels of morbidity and mortality. In this chapter we discuss the definition of malignant cerebral infarction and the role of decompressive craniectomy in the treatment of stroke and prevention of both morbidity and mortality.

In the United States, more than 80% of strokes are ischemic. Ischemic infarctions result from three main mechanisms: thrombosis, embolism, and cerebral hypoperfusion. Thrombosis typically is the result of disease of the arterial wall such as atherosclerotic plaque, whereas embolism is the result of vessel occlusion by blood clot or other material dislodged from a more proximal site such as a larger diameter vessel or a chamber of the heart. Thrombosis and embolism may lead to occlusion of vessels anywhere from the more proximal internal carotid artery (ICA) to the most distal branches of the intracerebral vasculature. Branches of the middle cerebral artery (MCA) are denoted with a number for each more distal branching point from M1 to M4. Each succession of branching vessels leads ultimately to an individual area of brain tissue perfused by the most distal vessels. Therefore, the more proximal a site of blockage is, the greater the amount of the parenchyma at risk for infarction. Systemic hypotension can lead to a more global process of damage with the greatest source of ischemic damage being in the areas of distal vessel overlap known as the "watershed zones" or "border zones" between the anterior cerebral artery (ACA), MCA, and posterior cerebral artery (PCA).

While a strategically placed stroke in the cerebral hemispheres or brainstem may lead to profound neurologic disability including loss of language abilities, alteration in personality, visual loss, and hemiplegia, few infarctions are associated with as high a likelihood of mortality or severe morbidity as are large artery occlusions that result in an infarction involving a large volume of brain. The "malignant" MCA syndrome is one of these infarctions, and typically is the result of occlusion of the distal ICA or proximal MCA [2]. Hacke introduced the term "malignant MCA syndrome" in 1996. It describes the small subset of stroke victims with severe hemiparesis and sensory loss, horizontal gaze deviation, and global aphasia (when the dominant hemisphere is involved) that progress to deterioration of their mental and respiratory status within the first 24 to 48 hours of symptom onset [2-5]. Death from cerebral edema and herniation leading to compression of the brainstem commonly occurs in the first 2 to 5 days following infarction [2, 3]. The malignant MCA syndrome is associated with a greater than 80% mortality [2, 6]. Fortunately, these infarctions are relatively rare, encountered in only about 10% of all patients with ischemic strokes [4]. Each year approximately 70,000 patients are at risk for the malignant MCA syndrome in the United States alone.

Predicting which patients will develop such severe stroke syndromes and their impending rates of death and disability is a source of great interest. Higher National Institutes of Health Stroke Scale (NIHSS) scores have a high sensitivity (96%) but a low specificity (72%) for

those patients with scores greater than 19 in the prediction of the development of malignant stroke syndromes, while identification on magnetic resonance angiography of an internal carotid artery “T-lesion” has a lower sensitivity of 64% but greater specificity of 85%, making neither a perfect predictor [7]. Imaging findings have been found to have the strongest predictive value. Early work by von Kummer et al. showed that early computed tomography (CT) hypodensity representing greater than 50% of the MCA territory within 5 hours of stroke onset was predictive of fatal outcomes in 85% of cases [8]. Later magnetic resonance imaging studies showed a 100% sensitivity and 94% specificity for predicting malignant MCA syndrome in patients with a diffusion-weighted imaging lesion greater than 145 mL up to 14 hours after symptom onset [3, 9]. Thomalla et al. demonstrated that more rapid completion of magnetic resonance imaging within 6 hours could be predictive of malignant MCA syndrome with changes in apparent diffusion coefficient volume greater than 82 mL [3, 7].

ACUTE MANAGEMENT OF ISCHEMIC STROKE

Time is of the essence in the acute management of ischemic infarction. Longer duration of ischemia leads to increasing neuronal death and likelihood of permanent damage. Treatment with intravenous tissue plasminogen activator (IV tPA) within 3 hours from the time of symptom onset has been shown to reduce morbidity at 3 months compared to placebo in the National Institute of Neurological Disorders and Stroke (NINDS) trial and then later with an extension of the time frame to 4.5 hours in the European Cooperative Acute Stroke Study 3 (ECASS III) [10, 11]. Data consistently demonstrate that for every 15-minute shortening in time to treatment there is a mortality and morbidity benefit [12]. The time standard of care for reperfusion treatment has been expanding since the positive results of trials for endovascular therapy were published beginning with the Multicenter Randomized Clinical Trial of Endovascular Treatment for Acute Ischemic Stroke in the Netherlands (MR CLEAN) [13] in January 2015. With the completion of additional trials, namely, Endovascular Treatment for Small Core and Anterior Circulation Proximal Occlusion with Emphasis on Minimizing CT to Recanalization Times (ESCAPE) [14], Extending the Time for Thrombolysis in Emergency Neurological Deficits - Intra-Arterial (EXTEND-IA) [15], Solitaire with the Intention for Thrombectomy as Primary Endovascular Treatment (SWIFT-PRIME) [16], and Randomized Trial of Revascularization with Solitaire FR Device versus Best Medical Therapy in the Treatment of Acute Stroke Due to Anterior Circulation Large Vessel Occlusion Presenting within Eight Hours of Symptom Onset (REVASCAT) [17], the American Heart Association/American Stroke Association (AHA/ASA) updated its acute stroke management guidelines to include use of endovascular therapy with stent retrievers for large artery occlusions (i.e., ICA and M1) for patients up to 6 hours from their last known normal time [18].

Ultimately, the goal of these interventions is recanalization of the occluded vessel to restore blood flow to distal ischemic tissue. Rha and Savers’ meta-analysis of 53 studies examining recanalization reported that recanalization was achieved 24.1% of the time spontaneously, 46.2% with IV tPA, and 83.6% with mechanical thrombectomy [19]. Recanalization is determined by several factors, the most important of which are length and

location of the thrombus. In MCA thrombosis there is a high likelihood of recanalization following IV tPA for clots less than 5 mm in length as compared to a less than 1% chance for clots longer than 8 mm [20]. Recanalization of the ICA has the lowest success rate at 49% across all methodologies, with IV thrombolysis yielding the lowest rate of 13% [19]. Even with the success of early recanalization, studies have shown that the final infarct volume, not recanalization, is what drives outcomes [21, 22]. Thus, mitigation of the morbidity and mortality associated with these infarctions depends not only on recanalization success but also on the subsequent management of patients at high risk for malignant cerebral infarctions.

STUDIES OF DECOMPRESSIVE CRANIECTOMY

After the emergent management and stabilization of acute stroke patients, including patients treated with IV tPA or endovascular recanalization, the patient is typically observed in a critical care or stroke unit. Patients thought to be at risk for development of the malignant MCA syndrome are usually observed in a critical care unit.

The studies discussed here were all designed to answer the question of the role of decompressive craniectomy in the treatment of malignant cerebral edema following ischemic infarction. Trials looked at the outcomes of mortality as well as modified Rankin Scale (mRS) scores [23] to quantify long-term disability, as described in Table 1. On this non-linear scale, 0 indicates no symptoms while 6 indicates death. The differences between scores of 3 and 4 are related to the ability to ambulate and attend to one’s bodily needs without assistance. These scores, which were found to have a significant relationship with discharge to institutional care in the large Nationwide Inpatient Sample data pool, are an active area of debate and discussion about how to judge the impact of the intervention [24, 25]. All trials were stopped early because of poor/slow enrollment and the desire to pool data for meta-analyses. These three trials form the basis of all the meta-analyses that will be reviewed later.

Table 1. Modified Rankin Scale scoring description

Modified Rankin Scale (MRS)
0 No symptoms
1 No significant disability, despite symptoms; able to perform all usual duties and activities
2 Slight disability; unable to perform all previous activities but able to look after own affairs without assistance
3 Moderate disability; requires some help, but able to walk without assistance
4 Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5 Severe disability; bedridden, incontinent, and requires constant nursing care and attention
6 Death

DECIMAL [26]

The decompressive craniectomy in malignant MCA infarction (DECIMAL) trial was completed at 13 stroke centers in France from December 2001 through November 2005. The trial enrolled patients between the ages of 18 and 55 within 24 hours of an acute ischemic stroke that met criteria suggestive of the patient's being at high risk for a malignant MCA syndrome. Patients were enrolled if they had an NIHSS score of greater than 16, CT of the head with hypodensity in more than 50% of the MCA territory, and a DWI stroke volume of greater than 145 mL. Thirty-eight patients were enrolled and randomized to receive either decompressive craniectomy within 6 hours of randomization or 30 hours following symptom onset versus maximal medical therapy which included preventing hyperthermia or hyperglycemia, maintaining the head of the bed to an elevation greater than 30°, intravenous hydration, and the use of intravenous mannitol in patients with rapidly progressing symptoms due to edema.

The results of this first study demonstrated a life-saving effect with respect to craniectomy. Those in the surgical arm had an almost 53% absolute reduction in death. Follow-up for patients was reported at 6 months and 1 year after enrollment. At both time points, a greater proportion of patients had a mRS of less than or equal to 3 in the surgical treatment group than the medical group. At 6 months the difference was 25% versus 5.6%, and at 1 year those percentages increased to 50% and 22.2% for surgical and medical therapy, respectively, as depicted in Figure 1; the P values for these data points were not statistically significant. When using mRS of less than or equal to 4 as the outcome measure, the difference at 1 year increased to 75% in the surgical arm versus 22.2% for the medical arm ($P = 0.0029$).

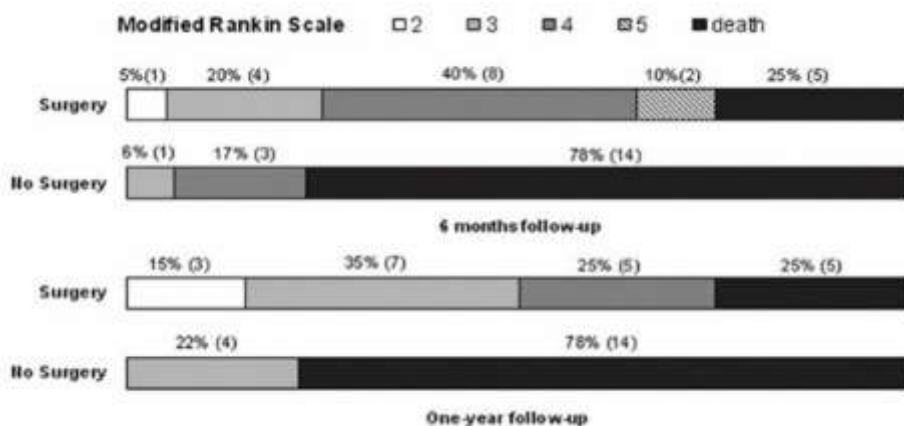


Figure 1. DECIMAL trial patient outcomes (Reprinted from *Stroke*, 38(9), Vahedi et al. Sequential-design, multicenter, randomized, controlled trial of early decompressive craniectomy in malignant middle cerebral artery infarction (DECIMAL Trial), Pg 2511, 2007, with permission from Elsevier).

The DECIMAL trial brought to light the idea that while survival was significantly improved by surgical intervention, the two treatment arms had no statistically significant difference in survivors with good outcomes (mRS less than or equal to 3). However, because the trial was stopped early, it likely did not have power to show a statistically significant difference.

DESTINY [27]

This trial of decompressive surgery for the treatment of malignant infarction in the MCA territory enrolled 32 patients across six study sites in Germany from February 2004 through October 2005. Inclusion criteria for this study included subjects aged 18–60 years, NIHSS score >18 in patients with non-dominant hemisphere infarcts and >20 when in the dominant hemisphere, and CT imaging findings demonstrating infarction of greater than two thirds of the MCA territory including part of the basal ganglia. Patients were randomized either to conservative therapy, which allowed for the use of osmotic therapy for any signs of increasing edema, intubation and hyperventilation, sedation, ICP monitoring, and maintenance of normothermia and euglycemia, or to surgical decompression within 36 hours of symptom onset.

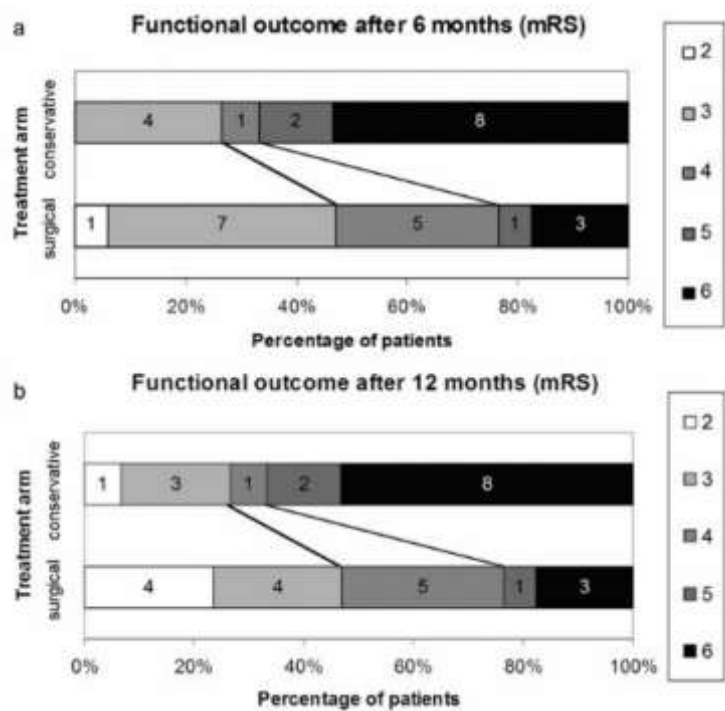


Figure 2. DESTINY trial patient outcomes (Reprinted from Stroke, 38(9), Juttler et al. Decompressive Surgery for the Treatment of Malignant Infarction of the Middle Cerebral Artery (DESTINY): a randomized, controlled trial, Pg 2523, 2007, with permission from Elsevier).

Randomization groups were fairly evenly matched, though it was noted that because of more patients with dominant hemisphere strokes in the control group, the NIHSS score was modestly higher at time of entry to the study in this group. Patients were predominantly screened and treated at one center. End points for the study included the 30-day survival reported as 88% in the surgical arm versus 47% in the conservatively managed group. The trial was designed to evaluate not only the mortality associated with malignant infarctions but also the functional outcomes at 6 months and 1 year, as represented in Figure 2. Again, the study results were dichotomized at a mRS score of less than or equal to 3, with 47% of

patients achieving this in the surgical group and only 27% in those conservatively managed. These results again trended positive but were not statistically significant, whereas when the good outcomes score is expanded to mRS less than or equal to 4 there is a significant improvement based on surgical intervention.

HAMLET [24, 28]

The Hemicraniectomy After MCA infarction with Life threatening Edema Trial (HAMLET) was completed at six centers in the Netherlands from November 2002 through October of 2007 and enrolled 64 patients. Patients aged 18 to 60 years were randomized to surgical decompression or medical therapy within 96 hours of symptom onset. The inclusion time of up to 96 hours from symptom onset was a major difference between HAMLET and the other trials (24 hours for DECIMAL and 36 hours for DESTINY). Inclusion criteria included a NIHSS score of >16 in right hemispheric stroke patients and >21 for left hemispheric stroke patients as well as hypodensity covering at least two thirds of the MCA territory on CT imaging.

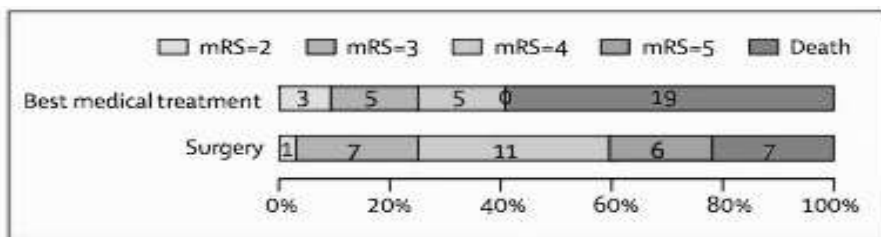


Figure 3. HAMLET trial patient outcomes (1 year) (Reprinted from The Lancet, 8(4), Hofmeijer et al. Surgical decompression for space-occupying cerebral infarction (the Hemicraniectomy After Middle Cerebral Artery infarction with Life-threatening Edema Trial [HAMLET]): a multicentre, open, randomised trial, Pg 329, 2009, with permission from Elsevier).

In this study, the only endpoint that was shown to have a significant reduction with hemicraniectomy was the likelihood of death, with an absolute risk reduction of 38%. The primary outcome of mRS dichotomized between 0–3 and 4–6 at 1 year had an absolute risk reduction (ARR) of 0%, though when extended to 0–4 versus 5–6 there was a trend toward benefit with surgical decompression and an ARR of 19%, as depicted in Figure 3.

Meta-Analyses

DECIMAL and DESTINY were halted prematurely in 2006 to combine the data for a meta-analysis. Each of the three trials had a similar protocol and outcome parameters allowing for relative ease of pooling patient data. Given the differences in eligibility criteria between studies, subjects were only included in the combined analysis with ages between 18 and 60, an NIHSS score >15, and a CT scan demonstrating infarction of at least 50% of the MCA territory or DWI infarct volume >145 mL. From these three studies, 93 patients were included who had been randomized within 45 hours of symptom onset [29]. In this data set, a

primary outcome of mRS 0–4 was chosen to define favorable outcome, with secondary analyses using mortality at 1 year and favorable outcome of mRS 0–3.

The pooled analysis demonstrated the positive effects of decompressive craniectomy with an ARR of 51.2% when comparing patients with mRS 0–4 versus 5–6 at 12 months [29]. Craniectomy reduced rates of death from 71% to 21.5%, as seen in Figure 4, essentially inverting the historical mortality rate for malignant MCA infarctions. In addition, when dichotomized for mRS 0–3 versus 4–6 there was still a significant ARR of 22.7% ($P = 0.014$). These data suggested that the previous concern that decompressive craniectomy improves survival while leaving the majority of patients in a severely debilitated state (i.e., mRS = 5) was false and that, in fact, the likelihood of this was nearly unchanged.

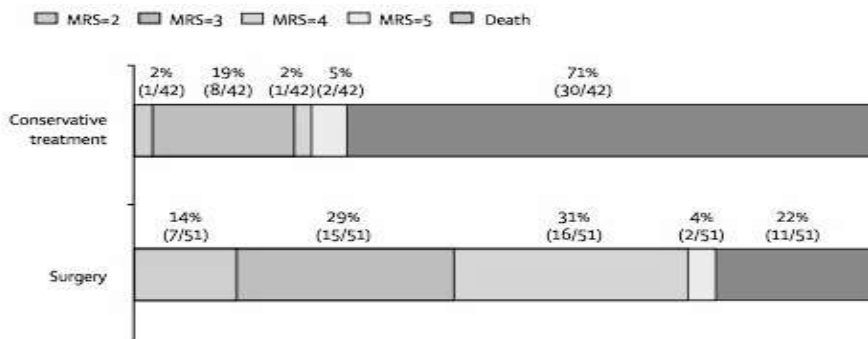


Figure 4. Pooled Analysis of DECIMAL, DESTINY, and HAMLET (1 year patient outcomes) (Reprinted from *The Lancet*, 6(3), Vahedi et al. Early decompressive surgery in malignant infarction of the middle cerebral artery: a pooled analysis of three randomised controlled trials, Pg 218, 2007, with permission from Elsevier).

In the years since this first meta-analysis and the results of the individual trials were published (2007–2009), several additional groups have looked at the data for hemicraniectomy in malignant cerebral edema related to MCA infarction. These studies again highlight the long debated question of the utility of surgical treatment in malignant stroke when the risk of severe disability is weighed against death and how severe disability should be defined.

Back et al. reviewed six studies in 2015 that included the three previously described studies as well as three more recently completed studies [30]. DESTINY2 [31], the follow-up to DESTINY, examined the effect of surgical decompression in patients older than 61 years. The Hemicraniectomy and Durotomy Upon Deterioration From Infarction-Related Swelling Trial (HeADDFIRST) [32] standardized the medical therapy provided to patients in neurologic intensive care units, including waiting until clinical deterioration before proceeding to decompression. Zhao et al. studied patients randomized up to the age of 80 in China [33]. To compare the 317 patients from these six studies, the researchers utilized odds ratios (OR) to assess the likelihood of each individual mRS outcome with respect to medical management versus surgery. They found that, as in each individual study, an mRS of 6 (death) was significantly reduced with surgical decompression with an OR = 0.19 and 0.17 at 6 and 12 months, respectively [30]. While the number of patients with mRS scores of 3 and 5 were increased at 6 and 12 months in the patients randomized to surgery, these did not reach statistical significance. The authors did find a significant increase in patients with mRS of 2 at

12 months (OR = 4.51). However, at both follow-up time points, surgical patients also had a statistically significantly higher likelihood of having an mRS of 4 with OR = 3.29 and 4.43 for 6 and 12 months, respectively [30].

Table 2. Likelihood of different outcomes based on meta-analysis of 6 trials [35]

	All patients		Patients >60 years	
	ARI	NNT	ARI	NNT
Excellent Functional Outcome (mRS 0-2)	6.6%	15.2	*	n/a
Favorable Outcome (mRS 0-3)	15.0%	6.7	4.0%	25.0
Unfavorable Functional Outcome (mRS 4-5)	29.5%	3.4	32.5%	3.1
*No patients > 60 with mRS 0-2				

ARI = Absolute Risk Increase; NNT = Number Needed to Treat.

Two additional meta-analyses have been performed by Yang et al. and Streib et al. utilizing five of the same six studies, but replacing the HeADDFIRST data with that from the preliminary results of a Croatian study by Slezins et al. [34, 35]. In the review by Yang et al. mortality was significantly decreased in the setting of early craniectomy (OR = 0.19, 95% confidence interval [CI]: 0.12–0.30; $P < 0.00001$), though the number of patients surviving with an mRS outcome of 4–5 was not statistically different for craniectomy or best medical therapy (pooled OR = 1.71, 95% CI: 0.78–3.74; $P = 0.18$) [34]. Both demonstrated that regardless of age the results were consistent, although in patients older than age 60 the likelihood of achieving survival with an mRS of less than 3 was zero regardless of treatment arm, and only 7% had a score of 3 after surgery compared to 3% in the medical arm [35]. In their primary analysis, Streib et al.'s meta-analysis evaluated the absolute risk increase for undergoing early craniectomy and therefore number needed to treat to meet defined functional outcome categories. Table 2 shows likely outcomes based on data from Streib et al. [35].

More recently, Dasenbrock et al. analyzed data from the National Inpatient Sample database to assess the predictors and association of time to decompressive craniectomy with outcomes. The review of approximately 1300 inpatient admissions evaluated the outcomes of discharge to institutional care (i.e., nursing facility, extended care facility, or hospice, but excluding rehabilitation or other acute care facility) and poor outcome (defined by death, tracheostomy and gastrostomy, or discharge to institutional care) based on time to surgery both on a continuous scale as well as dichotomously. Greater delay in surgery was associated with greater likelihood of discharge to institutional care with an OR = 1.17 (95% CI: 1.05–1.31; $P = 0.005$) and poor outcome with an OR = 1.12 (95% CI: 1.02–1.23; $P = 0.02$) (25). In the dichotomous analysis there was no difference in outcomes within 24 or 48 hours but increased odds of poor outcome after 72 hours (OR = 1.52, 95% CI: 1.07–2.16; $P = 0.02$). Discussion of the predictors for surgery concluded that craniectomy before herniation might be a more important factor, especially in patients who do not have significant decompensation prior to 48 hours from stroke onset [25]. Of course, this study also must be looked at with a cautious eye as the data collected were based on diagnostic coding as opposed to the more traditionally utilized data including NIHSS score, premorbid mRS, and distribution or size of infarct area. Still, the most interesting finding was that practices for timing of craniectomy did not seem to have any relationship to the year of operation as one might predict if the

previously discussed clinical trial data were being utilized as recommendations for changing clinical practice [25].

POSTERIOR CIRCULATION INFARCTIONS AND DECOMPRESSION

While a great deal of research has been directed toward determining the benefits of decompressive surgery in malignant MCA infarctions, there are limited data regarding aggressive treatment of patients with edema from cerebellar infarctions. Possibly more menacing than even large hemispheric strokes, those in the posterior circulation and affecting the cerebellum can lead to life-threatening upward herniation with only small increases in swelling due to the limited space in the posterior fossa. Suboccipital decompression (SDC) has been performed in the setting of brainstem compression and obstructive hydrocephalus as a life-saving therapy. Unfortunately, there have been no randomized controlled studies to suggest the actual benefit of this procedure nor research into which patients would likely benefit the most.

From March of 2007 until September of 2015, records of 1162 patients admitted to one of five hospitals in Korea for cerebellar infarction were reviewed and selected for a case-control study of SDC versus medical management. Twenty-eight patients who had undergone SDC and 56 who had not were reviewed and assessed using multiple logistic regression propensity score matching based on having had a cerebellar infarction volume ratio between 25% and 33% with a Glasgow Coma Scale score greater than or equal to 9 and no clinical deterioration prior to 72 hours from symptom onset. Insertion of an external ventricular device for cerebrospinal fluid drainage as treatment for hydrocephalus was performed in patients with findings of significant hydrocephalus, including 50% of those patients who underwent preventive SDC and 90.6% of those requiring emergent intervention [36]. At follow-up 1 year after the infarction, 66.7% of those receiving SDC had a favorable outcome ($P = 0.030$), defined as mRS of 0–2, versus 51% of those who had not undergone SDC ($P = 0.030$) [36]. The results from this case-control series indicate that *preventive* SDC prior to signs of brainstem compression or obstructive hydrocephalus in patients with an infarct volume ratio of 0.25–0.33 may be beneficial.

Even with the results of this study, there is still a great need for further research into the characteristics of those patients who have suffered a cerebellar infarction and will have overwhelming cerebellar edema leading to increased morbidity and mortality associated with brainstem compression or infarction. While not the main focus of this chapter, it certainly is an important area for future research.

QUALITY OF LIFE

Ultimately, each of these individual studies and meta-analyses demonstrates that while neurosurgical intervention has a clear survival benefit in nearly all patients with malignant MCA syndrome, the evidence of a beneficial difference in morbidity depends on the authors' definition of "good" outcome. Similar to the opinions espoused in the discussions of the more recent meta-analyses, we feel very strongly that a patient's individual beliefs and goals of

care need to be weighed when deciding to move forward with surgical decompression in the setting of malignant edema. The data suggest a trend toward an increased representation of patients with outcomes that would allow for a life at home, albeit many will require significant help from family members. Review of those same studies shows a statistically significant increase in the number of patients who survive with an mRS of 4 that would almost certainly mean a life relegated to living with dependence on others for all of their needs.

Quality of life (QoL) data were collected in most of these trials either prospectively or retrospectively and reveal that relatively few patients or family members regret the decision to undergo surgery despite the ultimate level of dependency [26, 37]. A review of seventeen studies of craniectomy in malignant edema reported outcomes for QoL with data available for six of those trials that looked at scores of overall QoL. Patient QoL measures were in a range of 60% to 67% (an outlier in the DESTINY study of 46% was noted) as compared to best possible QoL [38]. Depression among patients has been reported as mild to moderate in almost 50% of survivors and severe in 15% [38]. While these values may appear to be relatively high, in view of the fact that patients with more severe disability were also included in these analyses, it suggests that patient perception of disability may change or be different at baseline than previously believed [39]. This divide in the acceptability of outcome can be demonstrated when neurologists, neurointensivists, and neurosurgeons were polled: only 38% believed an mRS of 4 to be a favorable outcome [40]. Do patients simply adapt to a disability and life they never before would have accepted?

The answer to that question is exceedingly complex and requires an intimate knowledge of the patient's previous insights and life goals. As would seem obvious, most of the patients who present with a massive ischemic infarct were not expecting or planning for this abrupt change in their lives. Further complicating the situation is that many individuals have neither had a conversation with their legally authorized representatives nor documented their wishes in an advance directive. Even if a person had indicated their general wishes, the circumstances surrounding malignant brain infarction are unique enough that patients and family will need to extrapolate from their prior constructs to the specifics of their current medical condition. Moreover, the physicians caring for them did not have a prior relationship with the patient or their loved ones, though their primary care providers might be able to provide insight into the patient's wishes. It is our practice to look at each individual patient and discuss with the family and patient, if possible, the wide array of outcomes that may occur in the setting of potential malignant brain infarction. This conversation unfortunately relies on a great many unknowns and can be easily directed by the framework in which it is presented.

OPINIONS FROM EXPERIENCE

Reviewing the data from these trials suggests that in patients under age 60 decompressive craniectomy has both mortality and morbidity benefits, though the morbidity must be carefully defined as including the possibility of need for around the clock care. While none of the previously discussed studies analyzed data dichotomized by early versus later surgery, a study from China looked at "ultra-early" surgical decompression in those with an NIHSS

score greater than 20 and imaging suggestive of impending herniation syndromes at an inflection point of 6 hours. This study found a statistically significant reduction in mortality in surgically treated patients as well as compromised function as based on Glasgow Coma Outcome scores, with no increase in surgical complications if operated on before or after 6 hours from time of stroke onset [41]. Thus, we believe, pending new research findings, that patients requiring hemicraniectomy should be treated within 48 hours of symptom onset, and not after the patient begins to demonstrate findings of progression from malignant edema. That being said, we appreciate that the glyburide treatment studies published to date, GAMES and GAMES-RP, recommended decompressive craniotomy only after the patient exhibited symptoms and signs of deterioration (defined as Glasgow Coma Scale score ≤ 8 , increase of ≥ 1 on item 1a of the NIHSS score, new pupillary abnormalities, or less brisk response to pain) to have time to determine the efficacy of medical therapy alone prior to surgery [42, 43].

In our own experience, we find hemicraniectomy to be infrequently advised within an early timeframe in the hope that optimal medical therapy will allow some patients with large MCA territory infarcts to “sneak by” without surgery. Often, surgical options are explored only after it is thought that medical optimization has taken the patient as far as it can and by this point there are signs of impending or active herniation (i.e., decrease in consciousness, pupillary changes, or evidence of vascular compromise of the non-infarcted hemisphere). Does holding off on surgery lead to irreversible damage that ultimately leaves the patient with increased disability and a higher likelihood of being classified as an mRS 4 instead of a 3? Might earlier surgery spare brain tissue at risk for infarction or anatomical distortion as suggested by observations in study patients treated with glyburide for prevention of brain swelling as well as laboratory observations in rats [44-46]?

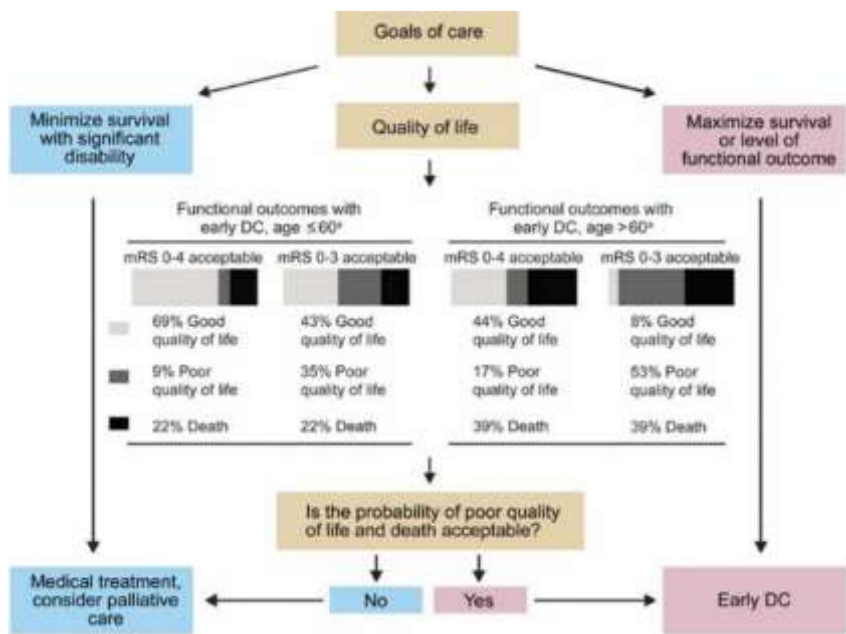


Figure 5: Algorithm for Hemicraniectomy Decision in Malignant Cerebral Infarction as developed by Streib et al. [35] (Reprinted from Neurology Clinical Practice, 6(5), Streib et al, Early decompressive craniectomy for malignant cerebral infarction: Meta-analysis and clinical decision algorithm, Pg 440, 2016, with permission from Elsevier).

There is an important role for early patient and family (or legally authorized representative) discussions to be conducted for patients at high risk for malignant infarction. To determine the most appropriate direction of care, a good understanding is necessary of what a patient may consider to be a reasonable outcome and in turn the care provider should describe the most objective outcome probabilities based on the data available. Streib et al. created a clinical decision algorithm that dichotomizes decisions based on whether a patient would find a life dependent on others acceptable. In their estimations for patients under the age of 60, when an mRS of 3 is considered acceptable QoL, there is a 35% likelihood of poor QoL with only a 43% likelihood of good QoL, whereas when an mRS of 4 is defined as acceptable, then those likelihoods are 9% and 69%, respectively [35]. For patients older than age 60, the likelihood of good outcomes is more restricted, as represented in Figure 5.

FUTURE DIRECTIONS

The meta-analyses discussed here are not the final statement for the care of patients with malignant MCA infarctions. The protocols demonstrate regional and individual physician treatment preferences, especially for medical management. In fact, there are now American Heart Association/American Stroke Association guidelines for medical management for patients at risk for severe brain swelling following stroke, though many of the interventions have not been critically evaluated [47].

Further research on and discussion of the ethics of decompressive surgery should acknowledge that decisions are often made within the context of time constraints and the stress of critical illness and are deserving of further exploration. The collaboration of stroke neurologists, neurointensivists, neurosurgeons, patients, and their families is paramount to the improvement in outcomes for patients with malignant edema from ischemic infarction. Ideally, an institutional protocol for directing conversation between disciplines should be developed and implemented, thus creating a pathway for this decision making process.

CONCLUSION

Large territory, “malignant,” ischemic infarcts are associated with exceedingly high morbidity and mortality. In the past 20 years, multiple studies [24, 26, 27, 29, 32, 33] have been undertaken to establish the benefits of surgical decompressive craniotomy. Results have been mixed demonstrating a paradoxical improvement in mortality and good outcomes, but at the cost of an increase in poor outcomes. Currently, there is no consensus with regard to the timing of surgical intervention and which patients will benefit. Review of the available literature [25, 29, 30, 34, 35, 37] suggests that surgery continues to be an individually applied treatment, though likely with the greatest positive impact on patients under the age of 60 and those who have decompressive surgery within the first 48 hours from symptom onset. Continued collaboration between neurologists, neurosurgeons, neurointensivists, and patients and their families to identify the patients whose clinical presentation and goals of care align with intensive therapy, including early surgery, is vital to providing the best chance for each patient to achieve a desirable outcome. Finally, should new brain anti-swelling drugs become

available, the concepts outlined will need to be re-examined within a new management context.

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Chapter 96

VIRTUAL REALITY-AUGMENTED REHABILITATION FOR PATIENTS IN SUB-ACUTE PHASE POST-STROKE: A FEASIBILITY STUDY

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ABSTRACT

Upper extremity (UE) rehabilitation is of utmost importance to the achievement of full inclusion and functional independence. Traditionally presented as well as technology-based therapeutic interventions have produced measurable changes in motor function and motor control but fall short of major reductions in disability. Animal models of stroke suggest that the first two weeks to one month post stroke may be a critical time period of increased brain plasticity. This study shows the feasibility of adding one hour of intensive robotic/virtual reality (VR) therapy to on-going rehabilitation in the acute phase of recovery post-stroke. All five of the subjects made substantial improvements in Upper Extremity Fugl-Meyer Assessment (UEFMA) scores (mean improvement = 6 points (SD = 2)) as well as improvements in Wolf Motor Function Test (WMFT) time (average decrease = 41% (SD = 35) after training with more consistent changes in the proximal

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arm portions of the WMFT and the UEFMA as well as in upper arm kinematics. Maps of cortical excitability indicate an increase in both the area of activation and the volume of activation of the first dorsal interosseous (FDI) muscle after a two-week training period.

Keywords: stroke, rehabilitation, upper extremity, arm, hand, virtual reality

INTRODUCTION

People post-stroke exhibit paresis of the upper and lower extremities. When a person with paresis uses their upper extremity (UE) for purposeful movement, the paretic movements differ from normal movements in that they are slower, less accurate, have delayed or reduced force, and are uncoordinated in terms of magnitude and timing of the movement. Upper extremity rehabilitation is of utmost importance to the achievement of full inclusion and functional independence. Plasticity-mediated therapies have evolved from the concepts of adaptive activity-based neuroplasticity, task-oriented motor training and the need for high doses of repetitive practice. One approach to plasticity-based therapy – the combination of virtual reality and rehabilitation robotics – has offered new promise for enhanced outcomes for upper extremity rehabilitation. The literature shows a progression of articles, from feasibility studies, to small clinical trials and a recent Cochrane review suggesting the potential benefits of using virtual reality for rehabilitation. However, to date, the best efforts of groups studying technology-based therapeutic interventions have produced measurable changes in motor function and motor control, but fall short of major reductions in disability.

The prevalence of studies in people with chronic stroke, or Cerebral Vascular Accident (CVA), stands in contrast to animal models of stroke that suggest that there may be a critical time period of increased brain plasticity (1). During the first month post stroke the peri-infarct cortex regains responsiveness to cortical afferents with accompanying increased dendritic spine morphogenesis and axonal sprouting (2). Rats trained with daily sessions of reaching showed significant gains in forearm reaching ability when the training was started 5 or 14 days post infarct but not 30 days post infarct (1). The important question these studies raise is whether early intensive training might be more effective.

To date only three studies have focused on task-based upper extremity interventions in persons in the acute period post-stroke. A study by da Silva-Cameriao of persons less than thirty days after CVA, identified faster recovery of upper extremity motor function in patients performing virtually simulated rehabilitation activities (VSRA) for twenty minutes in addition to a standard inpatient rehabilitation program (SIRP) when compared to controls who performed a dose matched increase in activity beyond their SIRP (3). This finding is important because it establishes the feasibility of using VSRA in addition to an SIRP very early after a CVA. This study also suggests that VSRA might have an effect during the acute stages of stroke. The second study using a more traditionally presented intervention (CIMT) did not demonstrate additive benefits (4).

This study was designed to test the feasibility of training patients between one and eight weeks after a CVA using our intensive robotically facilitated, virtually simulated UE motor intervention (5). The intervention utilizes several innovative, technology based approaches such as simulated activities that adapt in difficulty based on patient performance, robotic antigravity support, trajectory shaping as needed, and the virtual magnification of small active

finger movements into meaningful virtual activities. We hypothesized that this intense, targeted intervention would be tolerated well by persons in the first weeks following a stroke that were participating in inpatient or outpatient rehabilitation concurrently. In addition a battery of clinical, kinematic and neurophysiologic testing was performed to provide data to establish sample sizes for controlled studies of alternative treatments which will occur in the future.

METHODS

We performed feasibility testing with a single group of seven persons. Subjects were recruited from a consecutive sample of patients from the in-patient department of a suburban hospital. After initial screening by the department's physician, a physical therapist screened subjects based on the following criteria. Inclusion: (i) within 2 months post stroke; (ii) between the ages of 30 and 80; (iii) partial active shoulder flexion, or abduction, elbow extension and wrist extension against gravity; and (iv) trace extension at the fingers (detected visually) that can be reproduced several times in a minute. Exclusion: (i) severe spasticity (Modified Ashworth (4); (ii) cognitive deficits rendering them unable to follow three step commands or attend to task for at least ten minutes; (iii) hemi-spatial neglect rendering them unable to interact with an entire twenty four inch screen; (iv) proprioceptive loss that rendered a potential subject unable to interact with a virtual environment without looking at their hand; and (v) unstable blood pressure and oxygen saturation responses to activity. A separate screening and consent process for the motor mapping evaluation using transcranial magnetic stimulation was conducted.

System

The NJIT RAVR System consists of an instrumented glove combined with the Haptic Master (Moog-NCS, The Netherlands), a 3 degrees of freedom, admittance controlled (force controlled) robot. Three more degrees of freedom (yaw, pitch and roll) are added to the arm by using a gimbal for pronation/supination (roll). A three-dimensional force sensor measures the external force exerted by the user on the robot. In addition, the velocity and position of the robot's endpoint are measured allowing the robotic arm to act as an interface between the participants and the virtual environments (described below). The Haptic Master allows one to program the robot to produce haptic effects, such as spring, damper and constant force and to create haptic objects like blocks, cylinders and spheres as well as walls, floors, ramps and complex surfaces. The users interface with the Haptic Master via a forearm trough that extends through the gimbal, allowing for partial support of the weight of the arm as needed, while maintaining the ability to produce and measure pronation and supination movement. The NJIT TrackGlove System consists of a CyberGlove™ (Immersion, USA), an instrumented glove for finger angle tracking, and a TrackStar™ three-dimensional magnetic tracking system (Ascension Technologies).

Simulations

We have developed a suite of simulations for training shoulder, elbow, wrist and finger movements using the Virtools software package (Dassault Systemes). See Table 1 for descriptions of simulations that were used and the movement constructs they target.

Training protocol

To explore the feasibility of adding an hour of intense upper extremity activity to a standardized in-patient rehabilitation program, subjects received 60 minutes of training using the robot and the virtual reality gaming simulations in addition to their on-going in/out-patient physical, occupational and speech therapy for 5 days/week for two weeks.

Outcome measures

We examined the ability of our clinical, kinematic and neurophysiological measures to document changes in motor performance and neural control in persons having had their stroke less than thirty days prior to testing and training.

Clinical assessment. Subjects were tested one day prior to training (pre-test) and one day after training (post-test). A physical therapist performed the Wolf Motor Assessment Function Test (6) and the upper extremity portion of the Fugl-Meyer Assessment (7).

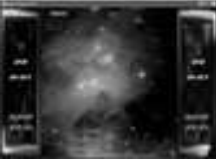
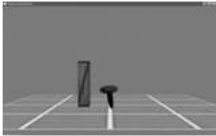
Kinematic assessment. Pre/Post kinematic measures included changes in distal kinematics, proximal kinematics and force. All measures were collected with the patients seated with their trunks supported and their hands resting on a table. Performance for all tracing tasks was measured following a single familiarization trial which was not scored.

Finger angles were collected using a Cyberglove™ (Immersion, USA). Finger range of motion was measured as the difference between all of the joint angles with the fingers in a relaxed/flexed position and the joint angles of all of the fingers actively extending the fingers as much as possible. Larger differences indicated better active finger extension range of motion. Finger Trace is the ability to modulate active finger extension and flexion between zero and eighty percent of max extension, measured by having the subject flex and extend their fingers to control a cursor tracking a sine function wave (period = 0.15 Hz, duration of 1 cycle $\approx$ 6 seconds). Lower root mean square error values indicate better performance.

Shoulder and elbow coordination was measured by having the subject trace a figure of eight (diameter of each circle approximately 20 cm, vertex at the midline of subject's body) on a low friction table top with their paretic hand. Lower root mean square error values indicate better performance. Hand path was measured with a TRACKSTAR™ three-dimensional magnetic tracker (Ascension, USA).

Pinch force was measured with an ATI Nano17™ force sensor (ATI Industrial Automation, USA). Pinch grip force is measured as the maximum voluntary force a subject can exert on a force sensor held between their paretic thumb and index finger, given two trials. Higher numbers indicate stronger pinch grip.

Table 1. Training simulations

Simulation	Training Goal	Game Play	Progression	Metric
Monkey Business 	Improve pinch grip force modulation	Subject controls monkey with pinch grip. Height that monkey jumps is in proportion to force of pinch grip. Subject makes the monkey jump to branches of varying heights.	Height of jumps are increased and time between jumps decreases as time to successful completion of the task decreases	Time/ Accuracy
Space Pong 	Finger Extension modulation	Subject plays a pong type game against the computer. Subject moves the paddle to the right by opening their fingers and to the left by closing them.	Decrease proportion of subject movement (finger extension) to paddle movement if accuracy does not improve with practice. Increase proportion when accuracy increases.	Accuracy
Space Ship 	Decreased arm elevation	Subject intercepts targets and avoids obstacles by piloting a spaceship with shoulder abduction and flexion movements	Increase target speed and obstacle density Increase workspace size as AROM increases.	Score increases with targets hit and obstacles avoided. Workspace size
Hammer 	Decreased Shoulder-Elbow Isolation	Subject hammers peg into the floor by pronating his forearm. Robot holds hammer stable over the target peg.	Decrease proportion of subject movement (pronation) to hammer movement as time to hammer pegs decreases	Peak pronation range of motion Time to hammer pegs
Piano 	Decreased Finger Individuation	Subject plays scales and simple songs. Each key is cued and the finger to press it designated.	Algorithm sets fractionation target based on performance. Utilize CyberGrasp™ to teach movement pattern if subject does not respond to algorithm.	Fractionation Time to press keys
Cups 	Decreased Shoulder-Trunk Isolation	Subject attaches virtual hand to virtual mugs and places them on virtual shelves in a 3-d workspace.	Increase volume of workspace as time to place cups on shelf decreases Recalibrate workspace weekly	Time to place nine cups on shelf Reaching trajectory length
Falling Objects 	Shoulder flexion towards midline	Subject moves a cursor to catch targets as they fall from the top of the screen. Targets drop centered on subject or in intact hemisphere.	Recalibrate workspace weekly.	Time to catch 100 targets Height of targets caught

Pinch Trace is the ability to modulate pinch grip force between zero and eighty percent of max pinch force, measured by having the subject vary pinch grip force to control a cursor tracking a sine function wave (period = 0.15 Hz, duration of 1 cycle $\approx$ 6 seconds). Lower root mean square error values indicate better performance.

TMS mapping. Subjects were tested one day before the therapy onset and one day after the end of the therapy. Subjects were seated with their arm, hand, and fingers comfortably secured in a brace to limit motion. To assure spatial TMS precision, each subject's high-resolution anatomical MRI was used to render a 3D cortical surface that is co-registered with the subject's head for frameless neuronavigation (Advanced Neuro Technology). Transcranial Magnetic Stimulation (TMS) (Magstim Rapid2, 70mm double coil) was used to determine the hotspot for the contralateral first dorsal interosseus muscle (FDI). The TMS coil was held tangential to the scalp with the handle posterior 45° off the sagittal plane. Following determination of the FDI hotspot, resting motor threshold (RMT) was calculated as the minimum intensity required to elicit MEPs > 50 μ V in the FDI muscle on 50% of 6 consecutive trials. Surface electromyographic activity (EMG, Delsys Trigno, 2 kHz) was recorded from the FDI muscles of the limb contralateral to stimulation side.

Mapping was conducted on the lesioned hemisphere of both subjects. All mapping was performed with the subject at rest and stimulation intensity set to 110% of the determined RMT (8). A 10 cm $\times$ 10 cm area surrounding the motor hotspot was marked using the neuronavigation software to provide consistent map boundaries. TMS pulses were delivered within the bounds with special attention paid to regions surrounding the hotspot territory. Real time visual feedback of the MEP time traces and neuronavigated coil position provided to the experimenter during testing maximized the map information obtained by allowing for increased density of points in excitable and border regions, with less attention given to far-away non-responsive areas (9). For each stimulation point we computed the following measures: (i) MEP as the peak-to-peak amplitude of the EMG signal 20-50 ms after the TMS pulse; and (ii) background EMG, calculated as the EMG signal in the 50 ms interval before the TMS pulse (2nd order Butterworth filter, 5-250 Hz band-pass, full-wave rectified, 20 Hz low-pass envelope). A threshold of 50 μ V was used to identify MEPs from background EMG (8). To allow comparisons across maps and sessions, MEP amplitudes and stimulation points were interpolated to a 10 cm $\times$ 10 cm mesh of 5 mm resolution centered on the M1 hotspot, using cubic surface interpolation (10, 11). Outcome measures include map area and volume, determined using double trapezoidal integration of the interpolated maps.

RESULTS

We performed feasibility testing with a single group of seven persons. Training was initiated an average of 20 (SD = 18) days post stroke. Average subject age was 63 (SD = 6.3) years (see Table 2). Please note that subject numbers correspond to the same patients for Tables 2-4 and Figure 1. One subject discontinued the protocol after being screened but prior to testing due to a second stroke, a second discontinued training after one intervention session due to fatigue. The five remaining subjects completed 8 sessions of sixty to seventy five minutes of interaction with a set of 6 simulated rehabilitation tasks in addition to completing 100% of their scheduled SIRP treatment sessions (90 minutes of PT, 90 minutes of OT and

45-90 minutes of ST per day) during the study period suggesting feasibility of this protocol. No adverse events were noted during VR training. All five subjects were able to understand the simulations well enough to participate without extensive instruction in spite of a relatively simple screening process (one three step command). Two of the seven subjects could not participate in TMS testing due to a history of seizures and three more did not consent to participating in this aspect of the study.

All five subjects made substantial improvements in UEFMA scores (Mean improvement = 6 points (SD = 2)) as well as improvements in WMFT time (average decrease = 41% (SD=35) (see Table 3). In addition, four of the 5 subjects completed at least one functional activity from the WMFT battery at post-test that they were unable to perform at pre-test. Four of the five subjects demonstrated improvements in clinical measures of proximal upper extremity function. In addition, 3 of the 5 subjects demonstrated improvements in the kinematic measure of proximal UE coordination (see Table 3). Distal UE function did not change as consistently. Only one of the five subjects demonstrated across the board improvements in all six measurements. Four of the five subjects demonstrated improvements in maximum pinch force. Four subjects demonstrated improvements in distal UEFMA (see Table 4). Figure 1 shows the changes in the distribution of the pattern of activation for the first dorsal interosseous (FDI) muscle acquired pre- and post- training for the two subjects from this sample that were medically appropriate, and who had consented to TMS testing. The MEP maps indicate an increase in both the area of activation and the volume of activation after training.

Table 2. Initial subject characteristics

Subject	Age	Gender	Time Since CVA(Days)	Hemiplegic side	Lesion Location	Initial UEFMA total
1	67	M	47	R	MCA	43
2	62	F	39	R	Pons	25
3	57	M	6	R	Pons	30
4	57	M	5	L	Parietal	24
5	74	M	12	L	Cerebellum	55

Table 3. Proximal UE clinical and kinematic measurements

Subject	WFMT Proximal (sec)		UEFMA Proximal		Figure 8 RMSE	
	Pre	Post	Pre	Post	Pre	Post
1	11.5	13.46	35	40	3.66	2.67
2	135.7	15.7	28	33	17.39	1.49
3	128.03	30.51	19	23	1.15	1.39
4	376.14	251.43	10	17	11.36	13.72
5	12.67	11.51	23	24	1.35	0.55

Table 4. Distal UE clinical and kinematic measures

Subject	WMFT Distal (sec)		UEFMA Distal		Finger Range (deg)		Finger Trace RMSE		Pinch Force Max (N)		Pinch Trace RMSE	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	671.06	672.63	20	24	23.55	10.8	4.69	7.21	19.91	18.16	5.74	6.84
2	960.00	845.82	15	27	12.64	37.5	3.30	1.32	1.43	8.74	0.60	0.33
3	375.94	405.89	11	13	41.77	38.6	3.51	4.32	16.28	26.36	3.84	3.14
4	847.22	735.87	14	13	57.89	49.9	7.28	7.75	21.02	30.78	5.53	5.99
5	144.46	50.74	2	7	51.43	56.2	11.3	10.31	12.33	50.54	4.07	12.10

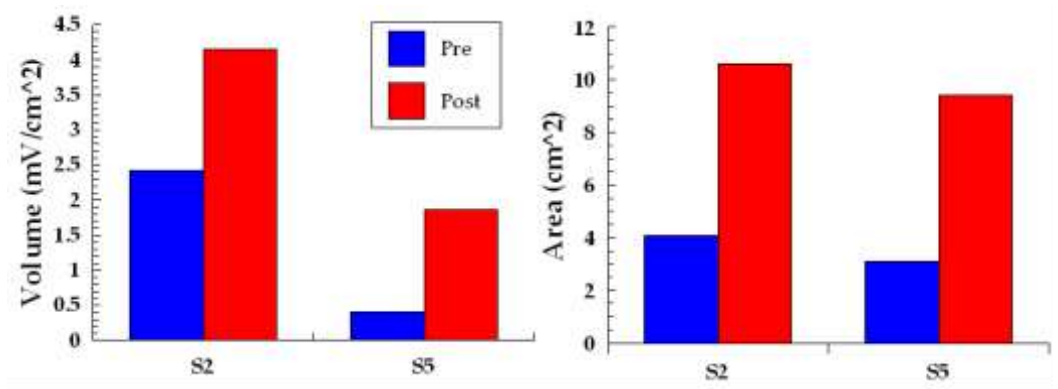


Figure 1. Volume and Area of TMS maps of first dorsal interosseus muscle of paretic hand of two subjects (S2 and S5) measured before and after training.

DISCUSSION

This study demonstrated the feasibility of adding one hour of intensive robotic/VR therapy to on-going rehabilitation in the acute phase of recovery post-stroke. Five of the seven subjects were able to participate in and tolerate the additional intensive activity with two of these five subjects beginning the robotic/VR therapy 5 days and 6 days post-stroke. Subjects vital sign responses to intense upper extremity activity remained stable throughout training. Subjects were supervised during 100% of the training sessions which is common for patients in the earliest phases of rehabilitation after a stroke.

This study elucidated several important issues regarding determining the efficacy of such early intensive intervention. As a group the subjects showed a 41% change in the WMFT after the two-week training period. This change is double what we have found when training patients in the chronic phase (22%). It has been demonstrated that the effect sizes reported for several clinical measures (e.g., the Frenchay, WMFT, TEMPA, ARAT, Jebsen) calculated at less than 3 months post-stroke were substantially larger than those calculated at three months or later post-stroke. The authors speculated that the observed differences in effect sizes during this time period likely reflect the inherent neuroplasticity early after stroke. However, when subjects’ outcomes were examined individually there was a noted variation in their response to treatment. Overall, there were more consistent changes in the proximal arm portions of the WMFT and the FM as well as in upper arm kinematics. Da Silva-Cameirao (3) reported a similar response on the recovery of proximal movements in a pilot study using the

Rehabilitation Gaming system in the acute phase post-stroke. This pattern of change was also noted in the kinematics of the movement. The changes in the distal clinical and kinematic measures were more varied with the exception of consistently robust changes in the maximum pinch force. The large changes in motor function, at least a portion of which will occur spontaneously, and the varied patterns of response in these subjects, would suggest that large populations will be necessary to test hypotheses effectively in this population.

An important biomarker of functional recovery is the excitability of the corticospinal system (e.g., the integrity of the motor output) (11, 12). Transcranial magnetic stimulation (TMS) induced motor evoked potentials (MEP) are an established proxy of corticospinal excitability. The initial data in this feasibility study suggest that in the days following stroke, training and recovery may be associated with an expansion of the corticospinal network (area) and strengthening of corticospinal synaptic weights (volume). This said, testing corticospinal excitability using TMS in acutely ill patients can be challenging. Less than a third of our subjects (two of seven) were medically appropriate for TMS and consented to participate. Based on our participation rate, studies planning to use TMS measures to test hypotheses in subjects with acute CVA will need to recruit from large pools of potential subjects.

In this feasibility study we have shown that providing an intensive robotic/VR intervention in the early phases post-stroke and integrating it with on-going usual care rehabilitation is a definite possibility. There are multiple challenges to this type of intervention, the inherent heterogeneity of stroke, the variation in the time course of spontaneous recovery and the confounding effect of neural restitution and compensatory functional movements. Improvements in motor performance are probably the result of several processes that occur in parallel to each other. We propose that our battery of clinical, kinematic and neurophysiological measures might be able to provide a window into the overlapping processes and help to sort out the confounding issues in the recovery of upper extremity function post-stroke.

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Chapter 97

**A COMPARISON OF UPPER LIMB MOVEMENT
PROFILES WHEN REACHING TO VIRTUAL AND REAL
TARGETS USING THE OCULUS RIFT:
IMPLICATIONS FOR VIRTUAL-REALITY ENHANCED
STROKE REHABILITATION**

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ABSTRACT

Recent innovations in the field of virtual reality, such as the Oculus Rift head mounted display, provide an unparalleled level of immersion in the virtual world at a cost which is rapidly approaching mainstream availability. Utilising virtual reality has been shown to improve many facets of the rehabilitation process, including patient motivation and participation. These systems however, do not enable the user to receive feedback when interacting with virtual objects, which may influence the movement profile of a patient. Therefore, to investigate how a virtual environment influences movements during stance, participants were required to reach to a real and a virtual target. Their movements were quantified using a motion capture suit, and a virtual target was generated using the

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Oculus Rift. The motions to both targets (real and virtual) were compared using a number of measures calculated to characterize the velocity profiles. Performance based indicators such as time and variance in movement were diminished when reaching to a virtual target, and movements executed in VR recruited more muscular segments than their real counterparts.

Keywords: virtual reality, oculus rift, reaching, virtual target

INTRODUCTION

Virtual reality (VR) systems have been demonstrated to be applicable to many forms of rehabilitation, improving patient motivation, and overall rehabilitation efficacy. Henderson et al. (1) found evidence that increased levels of immersion in a virtual environment provide advantages in upper limb motor skill reacquisition. They concluded that high immersion added meaningfulness to exercise movements, which increased the patient's motivation and participation in training. Rand et al. (2) demonstrated a significant increase in the usage of the affected upper extremities when using commercial video games as compared to traditional therapy. They found that providing patients with a more enjoyable and motivating experience resulted in higher frequency and intensity of movement – which is understood to be a primary contributor to the promotion of synaptogenesis, and therefore recovery (3).

The Oculus Rift is a ground-breaking VR device, capable of providing an unparalleled level of immersion in a virtual world. Combining the Oculus Rift with motion capture allows a user to see through the eyes of a virtual avatar, and interact with a virtual world using their movements. However, the display latency and lack of haptic feedback may cause disturbances in the velocity profiles when performing simple movements. Indeed, recent studies (4) have suggested that the use of head mounted displays (HMDs), such as the Oculus Rift, leads to a degree of postural instability.

Reaching to objects is a fundamental activity performed in daily life, and the ability to do so during standing is often diminished in the elderly or in persons with neurological disorders. During stance, there are significant restraints placed upon human voluntary movement, which are absent in the seated position, involving the complex coordination between movement and balance performed by the central nervous system (CNS) (5). The activation and recruitment of muscles when reaching to a real target is well understood (6), but the effect of a non-physical virtual target is unknown. In particular, it is unknown how much perceived support humans use when reaching to fixed targets to program their movement patterns. The use of VR enables a direct comparison between fixed and virtual targets in terms of the human movements produced.

Virtual reality provides an excellent opportunity to train these skills in patients, but the differences in movement patterns that are produced when moving in a virtual world are poorly understood. Research suggests that performance based indicators, such as time to complete tasks, are diminished whilst moving in VR (7), but the underlying kinematic differences causing these phenomena are unknown. This study aims to investigate the effects of VR on human movement, by comparing the underlying structure of movements (velocity profiles of the hand) during stance when reaching to real and virtual targets.

METHODS

The following subsections describe the method based on the basic procedure, the motion capture and virtual reality systems, as well as the specific data formats employed.

Procedure

Four healthy adult male participants, three healthy adult female participants, and one stroke-affected adult male participant were included in this study, each with varying degrees of experience in virtual reality.

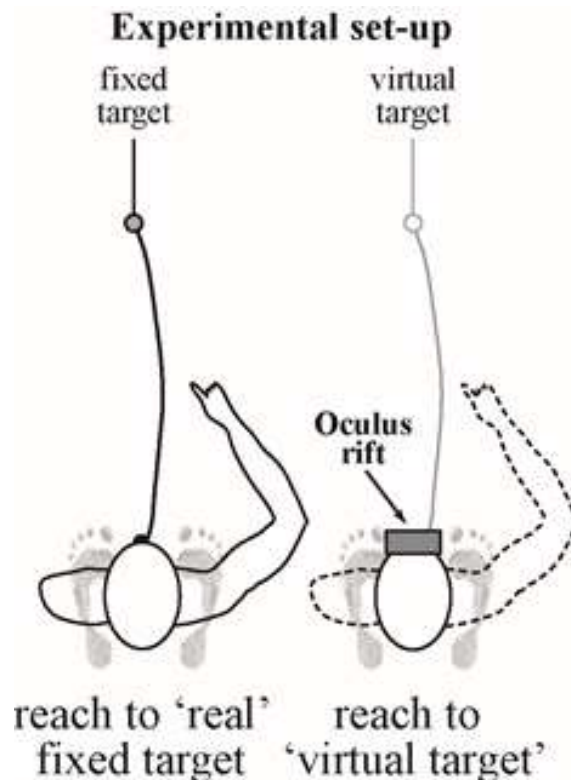


Figure 1. Reaching to real and virtual target.

A physical target was set up at 1.3 times the length of the person's reach when feet are flat on the floor. The participants repeatedly reached for the target with their preferred hand, held that position for 5 seconds, retracted their hand to their chest, and held that position for 5 seconds (non-VR Trial).

The same task was performed while wearing the Oculus Rift (VR-Trial). A virtual target was placed in an environment, which was calibrated to be exactly at the same location as the physical target by having the participant reach for the physical target, then setting the location of the virtual target to the in-game location of the hand. The physical target was then

removed. The only cue given concerning performance was the colour of the virtual target, which changed from green to red upon contact with the virtual avatar's hand (see Figure 1).

Motion-capture/VR system setup

Participants' movements were recorded using an XSENS MVN BIOMECH inertial motion capture suit. The suit was calibrated at the beginning of each session, to ensure that the readings for the real and the virtual tests were captured using the same calibration values. Recordings were captured by the associated software MVN Studio PRO, which provides the velocity data in x , y and z directions for 23 body segments at a capture rate of 120 Hz.

The motion capture data was streamed in real-time into the Unity game engine as a number of quaternion rotations, representing each segment of a 23 point kinematic map. This was then used to cause an avatar to reproduce the same movement.

The Oculus Rift HMD became the virtual eyes of the user. This HMD provided rotational information, which controls the orientation of the avatar's head in Unity, so that the user was able to freely look around the virtual world. Looking down, the user could see his/her virtual avatar's body, which was moving to match the person's own movements.

Quantifying the data

The motion data for each reach movement was exported from MVN Studio. Only the initial component of the reach movement to the target (fixed or virtual) was included, the motion data for return of the finger to the initial starting point being discarded. The beginning of the motion was specified as the point where the velocity magnitude of the hand reached 5% of the maximum achieved velocity.

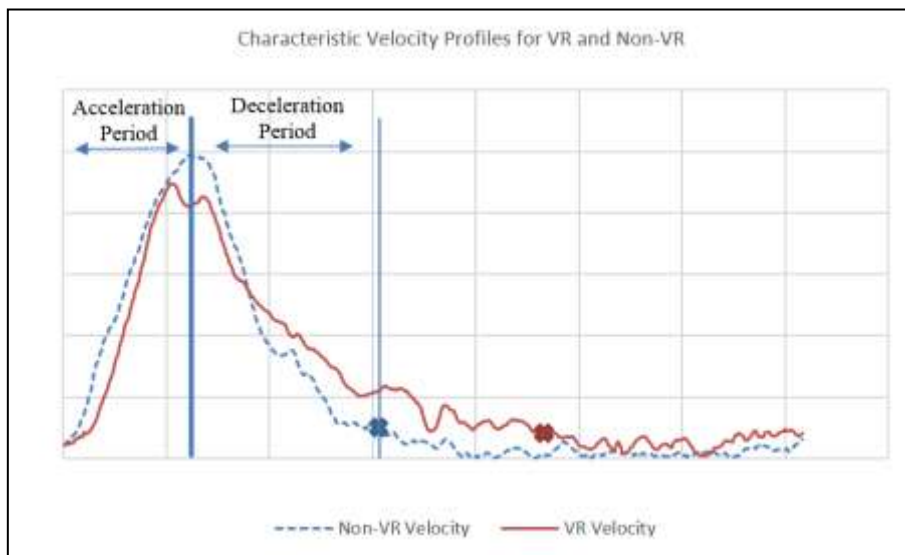


Figure 2. Velocity profiles of characteristic results, X marks where the target was reached.

All data sets exhibited a similar basic shape: a period of acceleration (activation) to maximum velocity, then deceleration (settling) towards the target (see Figure 2).

A number of variables were extracted from the data (see Table 1):

- 1) VMax – the maximum movement velocity.
- 2) ActGrad – the gradient for the acceleration phase: how quickly the maximum velocity was achieved.
- 3) Time to Target – the time used to reach the target.
- 4) Symmetry Ratio – the ratio between the acceleration and deceleration phases.
- 5) Settle MSE – the amount of variation in the settling segment.
- 6) Holding MSE – the amount of variation while holding position at the target.

Table 1. Non-VR vs. VR results for stroke-affected participant and average of healthy participants

	V _{Max}	ActGrad	Time To Target	Settle MSE	Holding MSE	Symmetry Ratio
Avg. Non-VR trial	0.94	0.08	1.48s	0.01	0.0007	0.68
Avg. VR trial	0.81	0.02	2.46s	0.04	0.003	0.29
Non-VR trial (stroke)	1.18	0.03	1.76s	0.06	0.001	0.26
VR trial (stroke)	0.99	0.02	2.97s	0.06	0.003	0.15

The velocity profiles recorded showed significant differences in shape between the VR and non-VR trials. The time to reach the target in VR trials took, on average, 0.64 seconds longer than non-VR trials for the healthy participants, and 1.21 seconds longer for the stroke-affected participant. The VR trials showed significant oscillation during the settling period (VMax to target contact) for the healthy participants, while the stroke affected participant showed a much higher level of oscillation, independent of whether it was a VR or a non-VR trial.

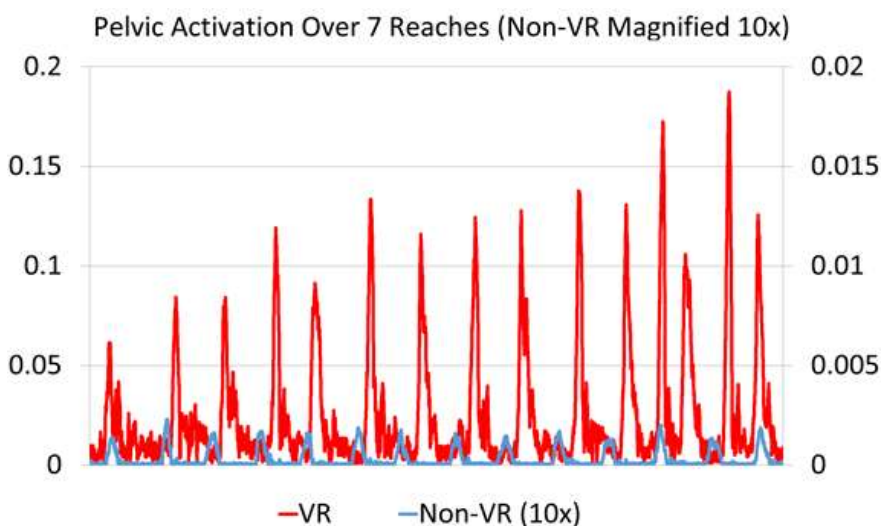


Figure 3. Pelvis velocity profile of 7 reaches in VR and Non-VR trial (Non-VR magnified 10x).

The MSE was calculated against a reconstruction of a stereotypical movement profile, created using the assumption that an ideal motion would accelerate and decelerate smoothly towards the target. The MSE calculated for all phases of the movement was significantly higher for the VR trials.

DISCUSSION

The results show that the movement profile of reaching to a virtual profile resembled that of reaching to a real target; however the deceleration phase took significantly longer, resulting in a longer time to complete the movement. The level of experience with VR did not seem to have a noticeable effect on the results, however this was the first time any of the participants had used motion capture in combination with an HMD. Participants did, however, seem to slightly improve their VR symmetry ratio over the trial, indicating that the subjects could possibly be trained through repetition so that their movements in VR more accurately resemble those in reality. This suggests that VR could potentially be used to enhance traditional movement retraining.

Upon inspection of the velocity profiles of other body segments, it was found that in general the VR movements recruited other segments significantly more than the non-VR movements. Figure 3 shows the velocity profile of the pelvis of one of the participants over 7 reaches. The non-VR movement registered almost no activation of the pelvis, while the VR movement shows significant pelvis activation. This result suggests that the muscular activation strategies employed in movements in VR are different to their real counterparts, and future research will be required to determine exactly how these activation strategies differ.

The fourfold increase in settling MSE indicated that subjects experienced uncertainty in the position of both their hand and the target, which validates previous research in virtual target acquisition (7). The lack of physical target to rest against for the 5 second hold accounted for the increased hold MSE.

CONCLUSION

Differences between the VR and non-VR trials were observed. In particular, reaching during standing to a VR target required a greater degree of control of the arm during the deceleration phase than reaching to a fixed target. These results must be considered in any further research into VR-enhanced rehabilitation, particularly when utilising HMDs such as the Oculus Rift, because moving in a virtual world does not produce the same movement patterns as moving in the real world. In general, actions in VR took longer to accomplish than their real counterparts, and displayed a higher level of variance in motion, indicative of a degree of uncertainty and instability. VR movements appeared to recruit more body segments in the movement than non-VR. Further study is required to determine if these phenomena are limited to subjects inexperienced with VR, which would mean that subjects could be trained and their movements improved through repetition. Whether this effect has an impact on the efficacy of motor rehabilitation remains to be seen.

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Chapter 98

SUBJECTIVE PERCEPTIONS WHEN USING MOTION TRACKING SYSTEMS: A COMPARISON AMONG HEALTHY SUBJECTS, INDIVIDUALS POST-STROKE, AND THERAPISTS

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ABSTRACT

Tracking technologies allow users to interact with virtual reality environments. Most research regarding tracking systems has focused on studying their performance parameters. However, even though subjective parameters also determine the responses evoked by the virtual reality experience, little effort has been made to study their influence. The objective of this paper is to determine the subjective perceptions of healthy subjects, individuals post-stroke, and physical therapists after using three tracking technologies (optical, electromagnetic, and skeleton tracking) to interact with a virtual rehabilitation exercise. Three groups participated in the experiment, involving healthy subjects (n = 19), individuals with stroke (n = 22), and physical therapists (n = 14). Participants belonging to the healthy and stroke groups interacted with a stepping exercise in three 15-minute trials using the three tracking systems. After each trial,

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participants filled in an ad-hoc questionnaire to report their subjective experiences. Physical therapists monitored 45 training sessions with the virtual rehabilitation exercise, 15 sessions with each tracking technology in randomized order. After the last session, they evaluated the three tracking systems completing another questionnaire. By and large, results showed that healthy subjects and physical therapists mainly preferred the skeleton tracking solution rather than the optical and electromagnetic solution (in that order). However, individuals post-stroke preferred the optical solution to the other options. In conclusion, subjective perceptions and preferences are far from being constant among different populations, thus suggesting that these considerations, together with the performance parameters, should be taken into account when designing rehabilitation systems.

Keywords: motion tracking systems, virtual rehabilitation, virtual reality, stroke

INTRODUCTION

Virtual reality (VR) can recreate synthetic environments that can be tailored to provide specific sensory stimulation in different channels. However to immerse individuals in an alternative reality, not only the stimulation is required, but also the virtual environment (VE) must react in a similar way to the real world, at least in certain aspects (1). Interaction with the VEs has been a technical challenge through the years. In order to detect and transfer the users' movements to the VE, different tracking solutions have been proposed. Tracking systems estimate the location and orientation of known targets with six degrees of freedom and transfer the data to the virtual world in real time (2). Traditionally, three main physical principles have been used to locate the targets, therefore classifying the tracking systems as either optical, electromagnetic, or inertial (or hybrid solutions combining the mentioned mechanisms).

Recent advances in technology have given rise to cheaper motion tracking solutions based on depth sensors, such as the Microsoft® Kinect™ (Microsoft®, Washington) or the ASUS® Xtion Pro (ASUS®, Taipei), both equipped with the PS1080 chipset (PrimeSense™ Ltd, Tel Aviv). According to the previous classification, these solutions can be considered as optical-based, because they estimate the depth information of a scene, but they are complemented with a statistical method to estimate the main joints of the human silhouettes present on the captured scene (3). Even though the classical definition of tracking systems requires the location of a target with six degrees of freedom, the location of the joints provided by the skeleton tracking is enough to interact with a great number of VE. The low cost of these devices, their comfort (no wearable sensors are needed), and their off-the-self availability have facilitated their widespread use (4, 5).

All the tracking technologies present different characteristics that are inherent to the physical principle on which they are based. Consequently, a tracking system can be defined by some parameters, such as accuracy, jitter, drift, and latency (2). Several studies have compared the performance of different tracking solutions according to these parameters (6-8). However, even though subjective considerations also determine the VR experience, thus modulating the immersion and presence of the users (9), limited research has focused on these aspects when using tracking systems. Interestingly, people with neurological impairments, as with individuals post-stroke, who are one of the target groups for VR-based rehabilitation

systems, may present sensory, motor, cognitive, and emotional impairments that can affect their interaction with the world (10, 11), and consequently, their subjective perceptions when using a tracking system.

The objective of this study was to evaluate the subjective perceptions elicited when using three different tracking technologies (optical, electromagnetic, and skeleton tracking) in three different populations: healthy subjects, individuals post-stroke, and physical therapists.

METHODS

Three different groups of participants were recruited. The age of healthy subjects and individuals post-stroke was matched.

The inclusion criteria in the healthy group were: (i) age ≥ 55 and < 80 ; and (ii) absence of previously reported motor or cognitive limitations. Individuals with previous experience with VR-based systems were excluded.

The inclusion criteria in the stroke group were: (i) age ≥ 55 years old and < 80 years old; (ii) chronicity > 6 months; (iii) absence of severe cognitive impairment as defined by Mini-Mental State Examination (12) cut-off > 23 ; (iv) able to follow instructions; (v) ability to maintain stride-standing position for 30 s without holding onto or assistance from another person as specified in the Brunel Balance Assessment, section 3, level 7 (13); and (vi) a Berg Balance Scale (14) score ≥ 41 . The exclusion criteria were: (i) individuals with previous experience with VR systems; (ii) individuals with severe dementia or aphasia; (iii) individuals whose visual or hearing impairment did not allow the possibility of interaction with the system; (iv) individuals with hemispatial neglect; and (v) individuals with ataxia or any other cerebellar symptom.

The inclusion criteria in the physical therapists group were: (i) physical therapy degree; and (ii) ≥ 2 years of experience in neurorehabilitation. Therapists with previous experience with VR systems were excluded.

Brief description of the tracking systems

Three different tracking solutions were used in this study: optical, electromagnetic, and skeleton tracking. The optical tracking consisted of two infrared cameras – the OptiTrack™ V100:R2 (NaturalPoint®, Corvallis) – aligned in the same plane (see Figure 1a). This setting allowed the location of spherical reflective markers present in the intersectional field of view of both cameras using epipolar geometry (15).

A G4™ (Polhemus™, Colchester) tracker was used as the electromagnetic solution (Figure 1b). Essentially, the tracking consists of an electromagnetic source and different sensors that are connected to a hub, which supplies them with power and transmits the tracking data to a PC. The sensors detect the electromagnetic field generated by the source and estimate their location and orientation (16).

With regards to the skeleton tracking, a Kinect™ and the Kinect for Windows SDK were used to track the body joints (see Figure 1c). This tracking solution estimates the depth

information of the scene, detects the human silhouettes present in the depth images, and applies a statistical method to fit a skeleton in the silhouettes (3).

A summary of the performance parameters of the tracking systems, principally defined by the manufacturer, is depicted in Table 1.

Table 1. Characteristics of the tracking systems

Characteristic	NaturalPoint® OptiTrack™ V100:R2	Polhemus™ G4™	Microsoft® Kinect™
Measurements(cm)	Camera: 7.5x4.5x3.7 Marker: 4 (diameter)	Source: 10.2x10.2x10.2 Hub: 10.6x1.9x6.6 Sensor: 2.29x2.82x1.52	Camera: 7.5x4.5x3.7 (5.8x28.2x6.8 with the support base)
Weight (g)	Camera: 119.1 Marker: 8	Source: 725.7 Hub: 114.0 Sensor: 43.0	Camera: 590
Frequency (Hz)	100	120	30 (with 1 skeleton)
Latency (ms)	10	10 (in optimum conditions)	150-500 (22)
*Resolution	RGB: 640x480 (at 100 Hz) with 8 bits	-	RGB: 640x480 (at 30 Hz) with 8 bits Depth: 640x480 (at 30 Hz) with 11 bits
*Field of view (°)	Horizontal: 46 Vertical: 35 (Default lens, 4.5mm F#1.6)	-	Horizontal: 57 Vertical: 43
*Wavelength (nm)	850	-	850
Connections	Wireless	Sensor-Hub: Wired Hub-Source: Wireless (proprietary RF link at 2.4 GHz with frequency hopping architecture)	Wireless
Power supply	Camera: 5 V, 490 mA Marker: Passive	Source: 5 V, 1 A Hub: 5 V, 500 mA (rechargeable battery) Sensor: Passive	Camera: 12 V, 1.1 A
Cost (\$)	1,198 (including 2 cameras)	5,250 (including 1 sensor)	249

*Resolution, field of view, and wavelength are parameters of the optical tracking systems.



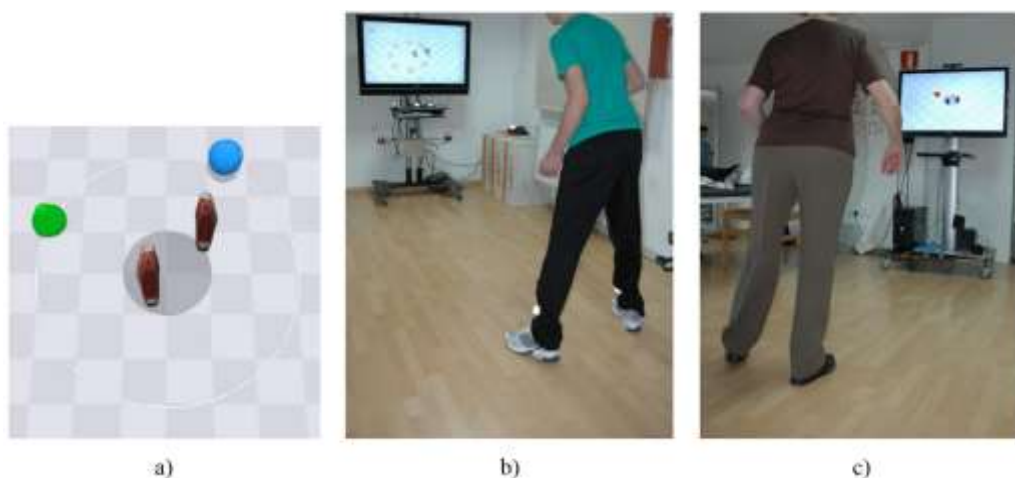
Tracking systems under study: a) optical; b) electromagnetic; and c) skeleton.

Figure 1. Tracking systems under study.

Virtual environment

A VR-based stepping exercise was used to assess the experiences of the participants of the three groups when using different tracking technologies (17). The VE consisted of an empty scenario with a checkered floor whose center was indicated with a darkened circle. The participants were represented by two feet that mimicked the movements of their own feet in the real world with a third person perspective (see Figure 2a). Initially, both feet appeared in the center of the circle. Different items rose from the ground in the surroundings of the circle, and disappeared after a few seconds. The objective of the exercise was to step on the rising items with the nearer foot while maintaining the other foot (the support foot) within the boundaries of the circle. After stepping on the items, the leg had to be drawn towards the body and placed within the circle to trigger the next target item.

The ankle joints (tibiotalar) of the participants were located and transferred to the VE by the tracking systems. In the optical and electromagnetic solutions, the joints were identified with reflective markers or electromagnetic sensors, respectively, fixed with a Velcro strip. The hardware setting of the VR system consisted of a standard PC, a 42" LCD screen, and one of the tracking systems described in the previous section (see Figure 2b and 2c).



Virtual reality based stepping exercise: a) snapshot of the virtual environment; b) participant interacting using the optical solution; c) participant interacting using the skeleton tracking solution.

Figure 2. Virtual reality-based stepping exercise.

Procedure

Three different VR units were installed in the physical therapy area of a neurorehabilitation center, each equipped with a different tracking system. The experiences of all the participants after using the three systems were collected through two ad-hoc questionnaires (A and B). Questionnaire A collected the experiences of healthy subjects and individuals post-stroke. Questionnaire B collected the experiences of physical therapists. The first four questions of both questionnaires evaluated the same topics.

Table 2. Scores of the subjective questionnaires

Issue	NaturalPoint® OptiTrack™	Polhemus™ G4™	Microsoft® Kinect™	Significance
Healthy, stroke individuals, and physical therapists				
<i>A1/B1. Fixation speed of sensors/markers</i>				
Healthy group	4.2 ± 1.0	4.0 ± 1.1	5.0 ± 0.0	O = G, K***>O,
Stroke group	4.3 ± 0.5	3.9 ± 0.6	4.4 ± 0.5	K***>G
Professional group	3.6 ± 0.8	3.2 ± 0.7	5.0 ± 0.0	O*>G, O = K,
				K*>G
				O = G, K***>O,
				K***>G
<i>A2/B2. Ease of calibration</i>				
Healthy group	4.5 ± 0.8	4.6 ± 0.7	4.8 ± 0.7	NS
Stroke group	4.3 ± 0.6	4.4 ± 0.5	3.0 ± 0.6	O = G, O***>K,
Professional group	4.1 ± 0.6	4.4 ± 0.5	3.1 ± 0.4	G***>K
				O = G, O***>K,
				G***>K
<i>A3/B3. Accuracy</i>				
Healthy group	4.7 ± 0.5	3.7 ± 0.9	4.3 ± 0.8	O***>G, O*>K*,
Stroke group	4.2 ± 0.7	3.9 ± 0.8	3.4 ± 0.7	K*>G
Professional group	4.6 ± 0.5	3.3 ± 0.8	4.0 ± 0.7	O = G, O*>K,
				G*>K
				O***>G, O*>K,
				K*>G
<i>A4/B4. Robustness</i>				
Healthy group	4.5 ± 0.6	4.7 ± 0.4	4.0 ± 0.8	G*>O, O = K,
Stroke group	3.9 ± 0.7	4.3 ± 0.7	3.4 ± 0.7	G***>K
Professional group	4.0 ± 0.8	4.6 ± 0.5	3.3 ± 0.8	G*>O, O*>K,
				G***>K
				G*>O, O*>K,
				G***>K
Healthy and stroke individuals				
<i>A5. Comfort</i>				
Healthy group	4.0 ± 0.7	3.5 ± 0.9	4.8 ± 0.5	O*>G, K***>O,
Stroke group	4.0 ± 0.5	3.3 ± 0.6	4.7 ± 0.5	K***>G
Professional group	-	-	-	O***>G, K***>O,
				K***>G
				-
Physical therapists				
<i>B5. Ease of fixation</i>				
Healthy group	-	-	-	-
Stroke group	-	-	-	-
Professional group	4.0 ± 0.6	3.4 ± 0.5	4.8 ± 0.4	O*>G, K*>O,
				K***>G
<i>B6. Insensibility to changes in the clinical setting</i>				
Healthy group	-	-	-	-
Stroke group	-	-	-	-
Professional group	3.1 ± 0.6	3 ± 0.8	3.7 ± 0.5	O = G, K*>O,
				K*>G
<i>B7. Ease of assistance</i>				
Healthy group	-	-	-	-
Stroke group	-	-	-	-
Professional group	4.1 ± 0.7	4.4 ± 0.7	2.5 ± 0.9	O***>K, G***>K,
				O = G

Issue	NaturalPoint® OptiTrack™	Polhemus™ G4™	Microsoft® Kinect™	Significance
<i>B8. Maintenance</i>				
Healthy group	-	-	-	-
Stroke group	-	-	-	-
Professional group	4.4 ± 0.7	3.3 ± 0.9	4.9 ± 0.3	O**>G, O = K, K**>G
<i>B9. Working range</i>				
Healthy group	-	-	-	-
Stroke group	-	-	-	-
Professional group	3.9 ± 0.8	3.2 ± 1.1	4.2 ± 0.7	O*>G, O = K, K*>G
<i>B10. Value for money</i>				
Healthy group	-	-	-	-
Stroke group	-	-	-	-
Professional group	2.5 ± 0.5	2.3 ± 0.7	4.8 ± 0.3	K**>O, K**>G, G = O
Healthy, stroke individuals, and physical therapists				
<i>A6/B11. Preference (n, %)</i>				
Healthy group	3 (15.8 %)	1 (5.2 %)	15 (79.0 %)	-
Stroke group	11 (50 %)	3 (13.6 %)	8 (36.4 %)	
Professional group	4 (28.6 %)	3 (21.4 %)	7 (50 %)	

Only significant differences are stated. K = Microsoft® Kinect™, O = NaturalPoint® OptiTrack™,

G4 = Polhemus™ G4™. Friedman with Wilcoxon as post-hoc. *p<0.05, **p<0.001. Significance: > higher than, = same as.

Participants belonging to the healthy and stroke group interacted with the stepping exercise in three 15-minute trials using the three tracking systems in counterbalanced order. The level of difficulty was determined by a physical therapist, who supervised all the sessions, to define an attainable but challenging task. After each trial, participants filled in questionnaire A. Questionnaire A consisted of six items to be assessed: (i) fixation speed of the sensors/markers; (ii) ease of the calibration; (iii) accuracy of the represented movements; (iv) robustness; (v) comfort; and (vi) order of preference. Responses to the first five items were rated on a 5-point Likert scale, where 1 means “very little/not at all” and 5 means “very much”. Responses to the last item were estimated as a percentage of preference.

The VR-based system was integrated in the physical therapy program. Patients who were attending a motor rehabilitation protocol in the neurorehabilitation center were assigned to train with the system according to their motor condition and expected benefits. Physical therapists monitored 45 training sessions with the VR-based exercise, 15 sessions with each tracking technology in randomized order. After the 45 sessions, the therapists, who were uninformed of the costs of the tracking systems during the entire study, were finally informed and filled in the questionnaire B for the optical, electromagnetic, and skeleton tracking. This questionnaire consisted of eleven items that assessed: (i) fixation speed of the sensors/markers; (ii) ease of the calibration; (iii) accuracy; (iv) robustness; (v) ease of fixation; (vi) insensibility to changes in the clinical setting; (vii) ease of assistance; (viii) maintenance; (ix) working range; (x) value for money; and (xi) order of preference. As in questionnaire A, the first tenth items were rated on a 5-point Likert scale and responses to the last item were estimated as a percentage of preference.

Statistical analysis

Demographical comparisons among groups were performed with independent sample t-tests and Chi-squared or Fisher exact tests, as appropriate. Repeated measures analyses were performed using the non-parametric Friedman test (χ^2 , p values) to determine within-group differences between tracking systems (NaturalPoint® OptiTrack™, Polhemus™ G4™, and Microsoft® Kinect™). When the Friedman test yielded a significant effect ($p < 0.05$), post hoc analysis was performed using a Wilcoxon signed-rank test for pairwise comparisons between tracking systems. The α level was set at 0.05 for all analyses. All analyses were computed with SPSS for Mac, version 20 (SPSS Inc., Chicago, USA).

RESULTS

After inclusion/exclusion criteria, the healthy group consisted of 19 individuals (12 males and 7 females, 60.8 ± 4.1 years old) and the stroke group consisted of 22 individuals (15 males and 7 females, 60.1 ± 7.0 years old). The stroke group included ischemic ($n = 11$) and haemorrhagic stroke ($n = 11$), and presented a chronicity of 272.4 ± 56.7 days. Of all the physical therapists working in the neurorehabilitation center, 14 therapists (6 males and 8 females, 31.8 ± 2.4 years old) satisfied the criteria and accepted to be included in the study.

Results of the three groups to the questionnaires showed that healthy subjects and physical therapists mainly preferred the skeleton tracking solution rather than the optical and electromagnetic solution (in that order). However, individuals post-stroke preferred the optical solution over the other options (Table 2).

DISCUSSION

Scores to the different items of the questionnaire are discussed below.

Fixation speed of sensors/markers. All the groups reported the Kinect™ as the least time consuming system, followed by the optical and the electromagnetic solution. Despite the significant difference between the skeleton and the optical tracking reported by the healthy and professional group (a difference in mean values of 0.8 and 1.4, respectively, respectively), individuals with stroke did not find this difference as relevant (0.1 difference in mean value). The fixation speed of the electromagnetic sensors was reported to be the lowest by the three groups. Interestingly, the professionals evaluated it with the lowest score, which can be explained by the fact that they also had to be careful with the position of the wires to avoid tangles.

Ease of calibration. No significant differences among tracking systems were reported by the healthy group. However, the stroke and professional group found the calibration for the skeleton tracking to be significantly more difficult than for the other systems ($p < 0.001$), since it required the participants to move to be tracked. This fact made the task more difficult for individuals with stroke, who presented motor impairments, as reported by clinicians and themselves.

Accuracy. The optical tracking system was reported to be the most accurate solution by all the groups ($p < 0.05$), consistent with the results of the performance study. The same

ranking order was found in the responses from healthy individuals and physical therapists. Individuals with stroke, however, reported the Kinect™ to provide the lowest accuracy ($p < 0.05$). The presence of motor impairments could have led individuals with stroke to execute irregular movement patterns and postures that could affect the body parts recognition and skeleton fitting processes.

Robustness. Similar conclusions can be inferred from the results of the robustness. All the groups defined the electromagnetic tracking solution as the most robust solution ($p < 0.05$), followed by the optical and the skeleton tracking system. Errors in the pose estimation could cause momentary maladjustments between the real and the virtual pose, which could be interpreted as a lack of robustness, especially by individuals with stroke and physical therapists, who reported the lowest scores (3.4 ± 0.7 and 3.3 ± 0.8 , respectively).

Comfort. The skeleton tracking system, which did not require sensors, was evaluated as the most comfortable solution by healthy subjects and individuals post-stroke ($p < 0.001$), followed by the optical and the electromagnetic solution. Differences between the optical and the electromagnetic tracking systems were also reported by the healthy ($p < 0.05$) and stroke ($p < 0.001$) groups. While the optical solution only required participants to wear reflective markers attached to their ankles, the electromagnetic solution also required them to wear a hub held to the waist of their pants, which was connected through wires to the sensors.

Ease of fixation. The professional group evaluated the skeleton tracking with the highest score, followed by the optical and the electromagnetic system (consistent with the scores in the fixation speed), which required therapists to fix the markers on the ankle joint of the patients. The electromagnetic solution, in addition, required the fixation of the hub and the careful placement of the wires in order to avoid tangles. The time and ease of fixation are crucial factors that must be minimized in clinical applications, where time is limited and should be dedicated to the physical therapy (18, 19).

Insensibility to changes in the clinical setting. The therapists considered that the skeleton tracking system was the least susceptible system ($p < 0.001$). However, the overall scores were low in comparison with other items. The optical solution was sometimes affected by reflections caused by chairs, room dividers, plinths, etc., elements commonly present in the clinical setting, or even by the sunlight. Even though these issues can be avoided by removing these elements from the field of view of the cameras or by closing the blinds, physical therapy units are dynamic areas where the spatial distribution is constantly changing and sunlight is appreciated. The electromagnetic tracking system proved to be the most susceptible solution to environmental changes.

Ease of assistance. The therapists reported that the electromagnetic tracking system was the solution that better allowed them to assist the patients. The physical principle of the G4™ made the performance of the system possible even when the therapists were between the source and the sensors. It allowed them to freely assist the patients from any position, and even to manipulate their extremities if needed. The optical tracking, on the contrary, required that the cameras had direct line-of-sight to the markers. The assistance, although possible, had to be provided from behind. Similarly, the Kinect™ required direct line-of-sight with the participants' complete silhouettes. Since the statistical method to detect the body segments was trained with isolated human poses, when therapists were close to the patients, manipulating or touching them, the system was not able to fit a skeleton to the resulting silhouette. Therapists had to hide from the view of the Kinect™ in order not to affect the tracking, which resulted in significant lower scores (2.5 ± 0.9 , $p < 0.001$).

Maintenance. With regards to maintenance, the therapists found that the need for recharging the hub of the electromagnetic tracking system after some hours of use was a limiting factor ($p < 0.001$). The other tracking solutions did not required special maintenance.

Working range. The professional group reported that the skeleton tracking system provided the largest working area, followed by the optical tracking system and the electromagnetic solution, which had significant lower scores ($p < 0.05$), consistent with the experimental results.

Value for money. The mass-produced Kinect™, which had the lowest price, achieved the highest score ($p < 0.001$). Scores to the other tracking solutions were also consistent with their price.

Preference. The healthy group mostly preferred the Microsoft® Kinect™ (79.0%), to the other tracking systems, which is consistent with their scores on the comfort item. Remarkably, this group did not experience significant problems when interacting with the system, as the Kinect™ is oriented towards healthy population. On the contrary, the stroke group mostly preferred the optical tracking system (68.2%). The issues mentioned derived from an incorrect skeleton fitting, which was more common in this group due to their motor restrictions, and which could have influenced their choice. These facts should be especially taken into account when working with individuals post-stroke, since they are likely to present behavioural problems (20), such as irritability or depression, which can make this population particularly prone to frustration and reduce the benefits of rehabilitation (21). In consequence, the use of the Kinect™ could be restricted to subjects with specific motor conditions. The therapists mostly preferred the skeleton tracking (57.1%), slightly over the optical solution (35.7%). This result could be explained as a trade-off between both systems. In spite the ease and speed of the Kinect™ startup and its ease of maintenance, the aforementioned issues with the Kinect™ can make the interaction of some patients difficult. The optical solution can overcome most of the interaction problems but it presents, however, some environmental restrictions (mainly light-related effects) that can affect its clinical use. An ideal situation allowing the use of these two tracking options, and the required space in the physical therapy unit, could satisfy all these requirements.

To summarize, our results show that subjective perceptions and preferences are far from being constant among different populations, thus suggesting that these considerations, together with the performance parameters, should be also taken into account when designing a rehabilitation system. In general, the skeleton tracking system was preferred by therapists and healthy individuals, and by a great number of individuals post-stroke. With regards to the therapists, though the assistance with skeleton tracking system initially posed a challenge for them (reported as the main issue of the technology), once they knew its functional limitations, they were able to provide assistance to the patients. This fact, together with its affordable cost, could have led them to finally adopt the skeleton tracking solution over the other systems, and can be the reason why they are using it currently in their daily practice.

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Chapter 99

EVALUATING THE MICROSOFT KINECT FOR USE IN UPPER EXTREMITY REHABILITATION FOLLOWING STROKE AS A COMMERCIAL OFF-THE-SHELF GAMING SYSTEM

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ABSTRACT

Motion controlled video games have been shown to have a positive effect for physical rehabilitation on the upper extremity in stroke survivors when combined with conventional physical therapy. The study investigates the therapeutic potential of the Microsoft Kinect for the Xbox360, a multimodal gaming peripheral. The Kinect allows users to interact with games using bodily motions and gestures. A list of important joint motions and movement synergies were identified by looking at leading stroke motor function tests for the upper limb. These have been verified by working with Occupational Therapists. A study group of occupational and physiotherapists were asked to record their experience of playing three Kinect mini-games and evaluate them with respect to their motor function requirements and exertion for each identified joint motion. Quality information was also gathered relating to the perceived usability and safety issues that could arise by presenting the device to a stroke survivor. The Kinect provides opportunities for gross arm movement exercise, while the requirement for highly raised

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arm movements will present a potential barrier for stroke users. Fine motor control movements of the hand and fingers are not tracked sufficiently for effective rehabilitation of the hand. A probable risk of falling while using the Kinect, and potential injury from overexerting the impaired limb while playing existing games, were also identified. We conclude that as the experience have been designed for able bodied users, the games present significant barriers for using the Kinect as a commercial off-the-shelf system for stroke rehabilitation.

Keywords: kinect, rehabilitation, upper extremity stroke, games, commercial off-the-shelf

INTRODUCTION

Stroke is the third most common disease in the UK with over 100,000 cases annually (1). Stroke is also the leading cause for long term disability (2). The cost of treatment and dealing with the long term disabilities afterwards is estimated to cost the UK economy £8.9 billion per year (3). Impaired arm function is a common effect of a stroke seen in around 70% of stroke cases; 40% of patients will have a completely non-functioning arm (4). Rehabilitation of the impaired limb is key to helping the stroke survivor regain independence. By allowing more stroke survivors to live independently the costs of providing disabled services and care can be reduced.

Physical rehabilitation is concerned with helping stroke survivors to regain control and coordination of the impaired upper limb. This involves direct contact with physical and occupational therapists to help the survivor develop and perform a set of exercises designed to help regain control in the upper extremity. Physical rehabilitation in this way can be limited by the therapist's availability; giving survivors more opportunity for rehabilitation increases the expected recovery (4). Following discharge from hospital the survivor may be required to travel to outpatient facilities for rehabilitation; this can be problematic for survivors with poor mobility (common following stroke) or who live in remote areas. The sudden and often unexpected stroke will have a significant effect on the survivor's life, making tasks that were once easy extremely difficult. It is therefore unsurprising that depression is common amongst stroke survivors. It is important that the survivor remains motivated and engaged with their rehabilitation especially in the early stages when the expected recovery amount is at its maximum (5).

Motion controlled video games have been shown to have a positive effect on physical rehabilitation of the upper extremity in stroke survivors when combined with conventional physical therapy (6-8). These games consist of an input method that requires movement of the upper extremity causing the player to exercise the impaired limb whilst playing the game. The game itself provides a motivational context that is fun and engaging for the player to interact with (9). While much research in this area has worked with bespoke systems and games, some research has been done into using commercial off-the-shelf gaming systems (COTS) for use in upper extremity stroke rehabilitation. As COTS systems are designed to be used in the home they offer the possibility of providing survivors with low cost systems that they can use to carry out rehabilitation at home (10). This could allow survivors to exercise at a level they are comfortable with, independent of physical or occupational therapists. It also offers a valuable opportunity to survivors who are unable to frequently travel to outpatient centres to

engage with rehabilitation activity. Low cost independent rehabilitation solutions may provide a cost effective way for long term rehabilitation which is currently limited by therapist resources (11, 12).

Historically the literature relating to COTS gaming systems for use in stroke rehabilitation has focused on two systems, the Playstation EyeToy and the Nintendo Wii. More recently however, following the launch of the Kinect system in late 2010, there has been a surge of interest in the investigation of the Microsoft Kinect and the role it can offer in this context.

The PlayStation EyeToy is a small camera that attaches to the PlayStation 2 gaming system. Functionally it is equivalent to a low cost USB web camera. The device outputs a low resolution colour image. The system then looks for any differences in colour at each pixel over time (between captured frames) to detect any motion at that pixel's location. As the system only looks for motion it is unable to distinguish between the player's movement and any background object. The system does not implement any pose tracking, so is unaware of the player's actual pose or position. To account for this the player is usually asked to stand or sit in a fixed position and use their arms to create the necessary motions. Rand et al. (13) tracked the scores and enjoyment of a group of stroke survivors and a group of healthy elderly users while playing several EyeToy games. Comparing the results showed that both groups found the experience enjoyable and highly motivating. However, the stroke group had significantly lower game scores; this can be accounted for by the stroke group reporting fatigue in the impaired limb, preventing them from playing as effectively. It is mentioned that the option to control the speed and difficulty of the games is not present but in this context would be desirable. Yavuzer et al. (14) reported a controlled, assessor blinded trial over four weeks where one group of stroke survivors took part in treatment using the EyeToy. The control group spent the same amount of time observing the game but not partaking. At the end of the four week trial the EyeToy group scored significantly higher when measured with the functional independence measure (FIM).

The Nintendo Wii uses a handheld controller device that can detect its position relative to a sensor bar mounted on the display device and the acceleration force on the controller. Like the EyeToy, the Wii is not able to perform body pose estimation as it can only gather positional information of the controller device. For most games this is assumed to be in the user's hand so can therefore be used to determine hand position. Joo et al. (15) found a slight increase in Fugl-Mayer assessment scores following two weeks of physical therapy using the Nintendo Wii. All subjects found the games enjoyable or very enjoyable. Problems were encountered during the study as some subjects had trouble holding the Wii controller due to poor hand motor control, therefore a custom strap was used to allow the user to 'wear' the controller rather than hold it. Saposnik et al. (16) compares using the Wii to conventional recreational therapy, finding that the Wii offers significant improvement in motor functions measured with the Wolf motor function test; Mouawad et al. (17) also found similar positive results.

The Microsoft Kinect for the Xbox360 is a multimodal gaming peripheral. Its primary input method is a novel low cost ranging (depth) camera that is used to drive a full body skeletal pose estimation system. This allows users to interact with games using bodily motions and gestures. Unlike other current motion controlled gaming systems the Kinect is marker-less so does not require the user to hold or wear any peripherals. The ranging camera allows position estimation in three dimensions allowing complex body poses to be tracked.

The accuracy and consistency of the Kinect sensor has been evaluated both in terms of its ability to measure the position of objects (18, 19) and upper body joint rotations (20). These studies suggest that it performs very well. It is however important to acknowledge that under certain circumstances use of the peripheral can be problematic. Some of these limitations are inherently a property of the technology itself. The use of projected infra-red light precludes the use of the sensor in areas of bright natural sunlight and, being camera based, occlusion is a big issue, particularly where the hand or arm pose is unconventional due to a disability.

Lange et al. (21) utilised the Kinect in a trial for balance training of adults with neurological injury. This study highlighted the difficulty of achieving the “calibration pose” necessary for the skeletal tracking to initialise properly. Of the twenty participants in this study, eight were unable to achieve this pose and the remaining twelve required the assistance of a clinician to manipulate the upper limb into a position suitable for calibration. Pastor et al. (22) also noted the problems with supporting the weight of an impaired upper limb, utilising the Kinect in a downward pointing manner so that a table top could provide support. These studies were based upon bespoke software solutions. The current crop of COTS games have the added drawback of also requiring the player to stand while playing, further exacerbating the potential usability issues associated with using the system and raising further questions about whether it has any potential as a COTS device for home rehabilitation. However the study conducted by Chang et al. (23) concluded very favourably in terms of the utility of the device, noting a significant improvement in motor function as well as recording high levels of motivation and recommending further investigation.

In this paper we present our findings from a study to evaluate the Kinect for safety and suitability as a COTS gaming system for stroke rehabilitation. The study also sought to identify areas that a bespoke system based upon Kinect hardware could improve upon, thereby enhancing, the rehabilitation possibilities beyond those available within existing commercial titles. The study’s focus was on identifying exercises of interest to upper extremity rehabilitation that are encouraged and facilitated by the system. Full upper extremity rehabilitation requires multiple exercises across a number of joints in the upper extremity. It is important to identify which regions the Kinect is effective at exercising so that its potential as a tool for rehabilitation can be assessed, as well as evaluating the usability of the Kinect device within the physical limitations of a stroke user group. The Kinect system is a full body skeletal tracking system. Games designed for the Kinect are built with able bodied users in mind, therefore as noted by Lange et al. (21) only a subset of available games, or game elements may be usable by a stroke user group with limited physical capabilities.

Safety concerns surrounding usage of the Kinect with a stroke user group were also explored. Stroke survivors with upper extremity deficits commonly have accompanying lower extremity problems, affecting balance and mobility. This can put the stroke survivor at a greater risk of falling. Therapists were asked to assess the presented games to identify if they would put a stroke user at a risk of falling. They were also asked to assess if the Kinect encourages exercises that could have a negative effect on the stroke survivor’s recovery.

As Xbox Kinect has over 140 games from first and third party developers only a small number of these could be included into the study due to limited resources and practicality of the study duration. Potential games were examined against the following criteria:

- Short experiences that a user is able to learn and play quickly are desirable considering the time constraints of the study.

- A complete round of a game should take no more than a few minutes to complete.
- The game must provide adequate instructions for a non-gamer to easily understand and play.
- The game must remain playable for a user with a low level of physical ability.



Figure 1. Kinect Sports for the xbox360 ©Microsoft 2010.

Using these constraints the game ‘Kinect Sports’ was selected. It is perhaps interesting to note that “sports” was a theme requested by participants in the Lange et al. study (21). Kinect Sports (see Figure 1) is a series of mini games based on real world sporting activities. As such the rules of the games are easy to learn even to non-gamers as they match the real world counterparts. The mini game format allows short game experiences to be played quickly and repeatedly without spending a significant amount of time operating menu systems. From a selection of six mini games, two were selected: Bowling and Table Tennis. These were chosen in order to compare a game that requires the user to react in a limited time (Table Tennis) with one where the user is given time to cognitively process and plan the activity (Bowling).

METHODS

The study was conducted over several repeated sessions with different occupational therapists. Therapists were allowed to participate individually or in pairs. When in pairs, each participant was required to play at least one round of each game individually, while the other

observed. Individual participants would watch a demonstration round provided by the session conductor. This was designed to allow participating therapists to observe the system from a first and third hand perspective.

At the beginning of each session the occupational therapists were given a brief demonstration of the Kinect system by an experienced user leading the session. The session conductor demonstrated how to start the Kinect system, navigate to the first game to play, start the game and give a brief demonstration of how to play the game. The system was then restarted and each occupational therapist in turn was asked to perform the start-up steps and play the game for several minutes including at least one full round of the game. After each occupational therapist had a chance to use the system and experience the game they were presented with a questionnaire to gather feedback from their experience. Participants were allowed to continue playing the game during the questionnaire. This process was repeated for each of the two mini games.

Population

The purpose of this study is to primarily evaluate if the Kinect is safe for use with a stroke group. Therefore no stroke survivors were involved at any stage of this study. The study population consisted of qualified occupational and physical therapists with experience of working with a stroke population. Six participants were recruited for the study. All six participants completed the study.

Data collection

Data was collected by questionnaires completed by the participants. The questionnaire asked participants to rate the minimum joints functionality required at each joint needed to meaningfully participate in the game, how exerting the exercise on each joint was during play and how much they felt the game encouraged them to use individual joint movements.

Participants were also invited to discuss their experience of playing each game. They were asked to focus on any potential safety issues they could foresee when presenting the Kinect system to a stroke population.

RESULTS

To visualise the results they have been graphed to show the mean score, standard deviation and range of the answers given. Of the two games tested, bowling was found to be more suitable for a stroke survivor as it required less arm and shoulder functionality to play. As shown in Figure 2, bowling required a moderate to high level of shoulder mobility to play, a moderate amount of elbow movement and very little wrist and hand movement. Comparatively table tennis, as shown in Figure 3, is far more intensive and requires a much higher level of motor functioning. This could be due in part to the time limitations of each activity. Bowling allows the player to take an indefinite amount of time to bowl each ball. Table tennis by contrast requires the player to make timed reactions to the computer

opponent. Cross body movements were required to perform backhand shots, this probably explains the increase in the amount of high intensity shoulder movements required.

Both games required little to no hand and wrist movement. This fits with our understanding of how the skeletal tracking algorithm works as it is unable to track wrist rotations and hand pose. Therefore we would expect games not to require wrist and hand movement as they are unable to detect them. Therapists did however feel that the games encouraged a greater range of joint movements than the system required. As shown in figure 4, therapists found themselves using hand and wrist motions when playing. As the games mimic real world activities the players may feel the need to match the normal movements even if they are not required by the Kinect games. In bowling, therapists found themselves making hand grip and release movements when picking up and throwing the ball.

By encouraging joint movements in this way the games could provide a wider rehabilitation benefit to more joints in the arm than are traceable by the Kinect camera. However as the joints are not tracked there is no guarantee that the user is performing them. This may be useful in a supervised therapy session where the therapist can observe the patient's movements. However in an independent usage situation there would be no record of the patient's hand movements and would therefore be of reduced usefulness for telerehabilitation.

Therapists were also asked to rate how intensively they were using each joint during play, as shown in Figure 5. Most joint movements were found to be low to medium intensity. This is good as many stroke survivors will have a weakened upper limb and may fatigue quickly when performing intensive tasks. Shoulder flexion and extension movements were found to be very intensive. This is because the game requires the user to make a large swinging movement at the shoulder. This is problematic for a stroke survivor as many will not have the required range of motion. This could present a barrier that they are unable to overcome and thus not be able to use the game.

Qualitative findings

Therapists were asked to provide additional feedback about their experience. Common feedback included raising concerns about the balance risks while using the device. As the game does not work in a seated position, players must be standing. Many stroke survivors have reduced mobility, placing them at an increased risk of falling. Because of the motor impairments stroke falls can be extremely dangerous as often the victim is unable to break the fall using their upper limb. This makes the Kinect a potentially dangerous system to use for rehabilitation, especially when unsupervised. Several therapists also expressed the desire for seated play as many survivors are unable to stand unaided so could not use the system. When developing bespoke games, allowing them to be played in a seated position should be considered a high priority.

Concerns over the game's graphical interface were also raised. Kinect Sports is designed to mimic sporting television coverage. This includes fast camera cuts and pans; detailed environments including crowds and decorative lighting; and statistical overlays that appear periodically. Cognitive deficit can accompany motor deficit during stroke, and many stroke survivors will not be familiar with video games. Efforts should be made to provide a clearer graphical interface.

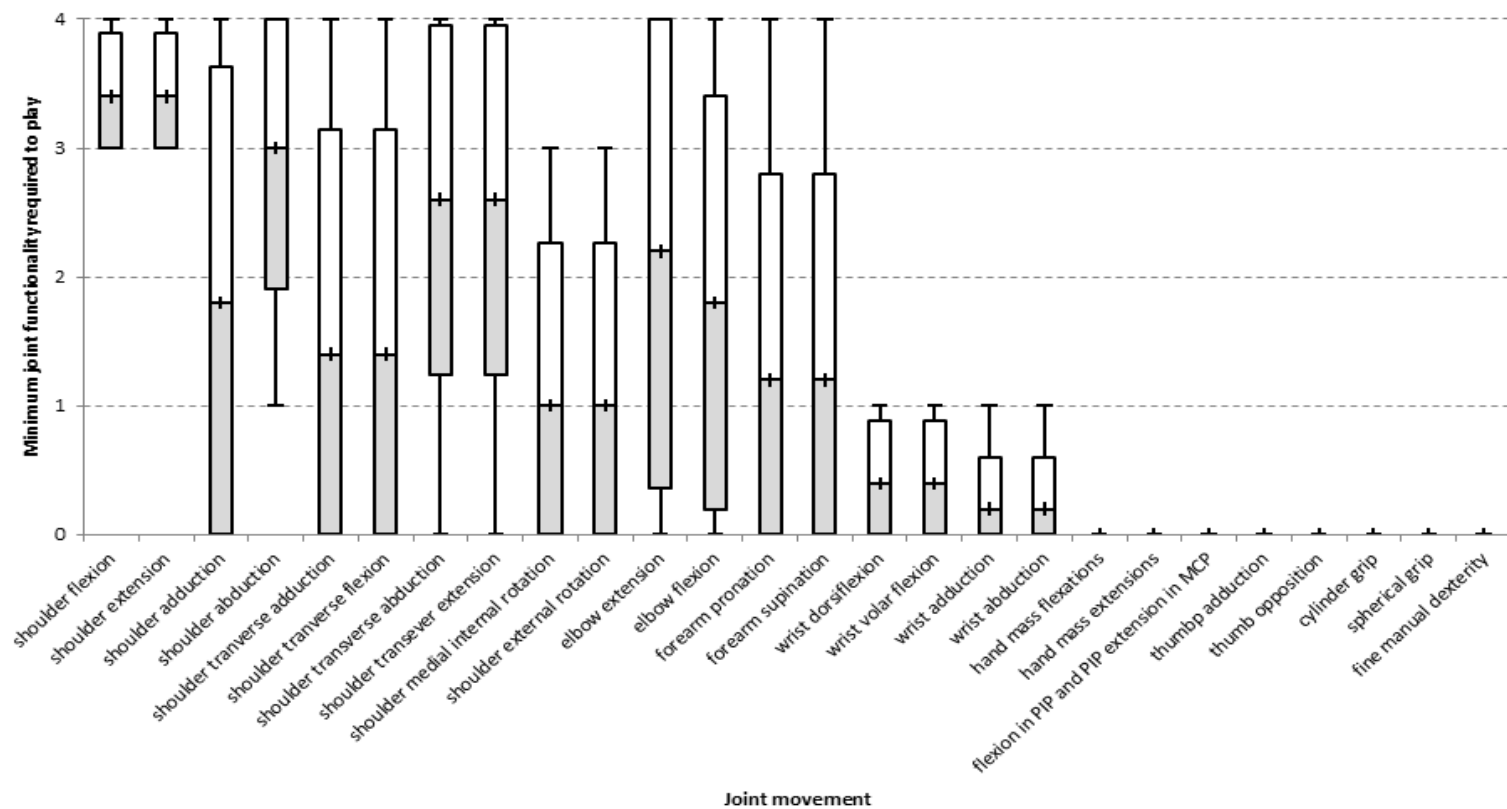
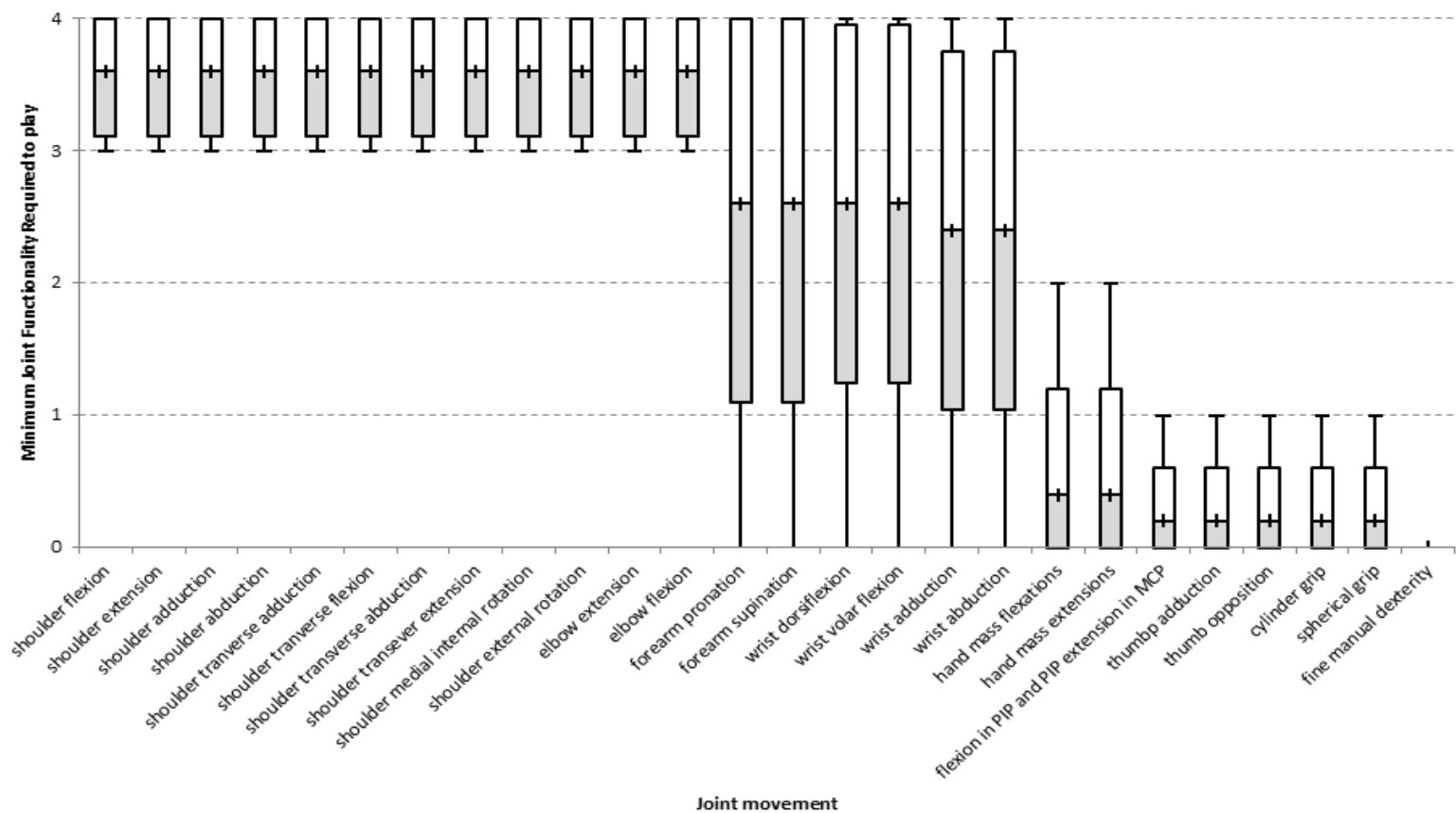
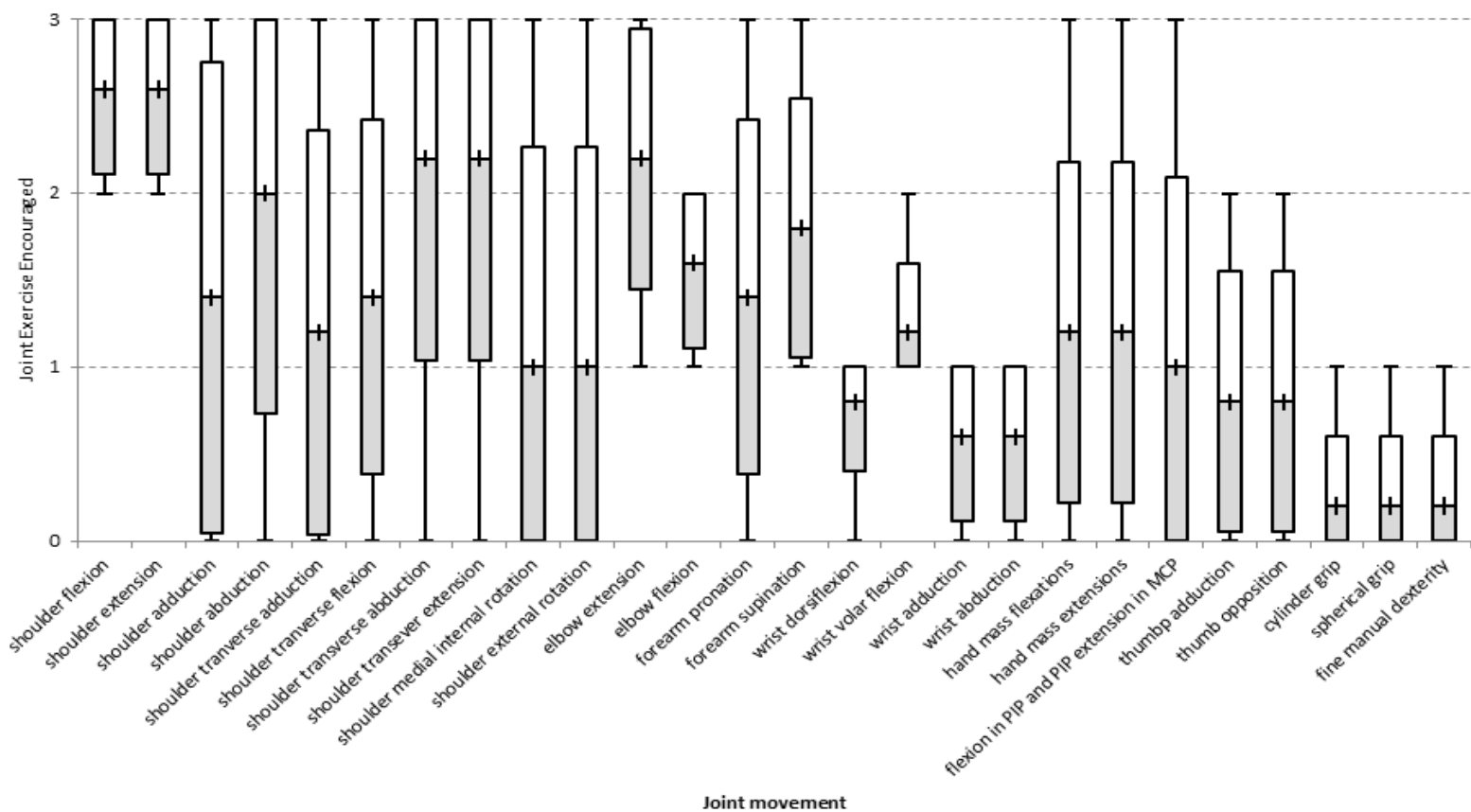


Figure 2. Minimum joint functionality required to play bowling.



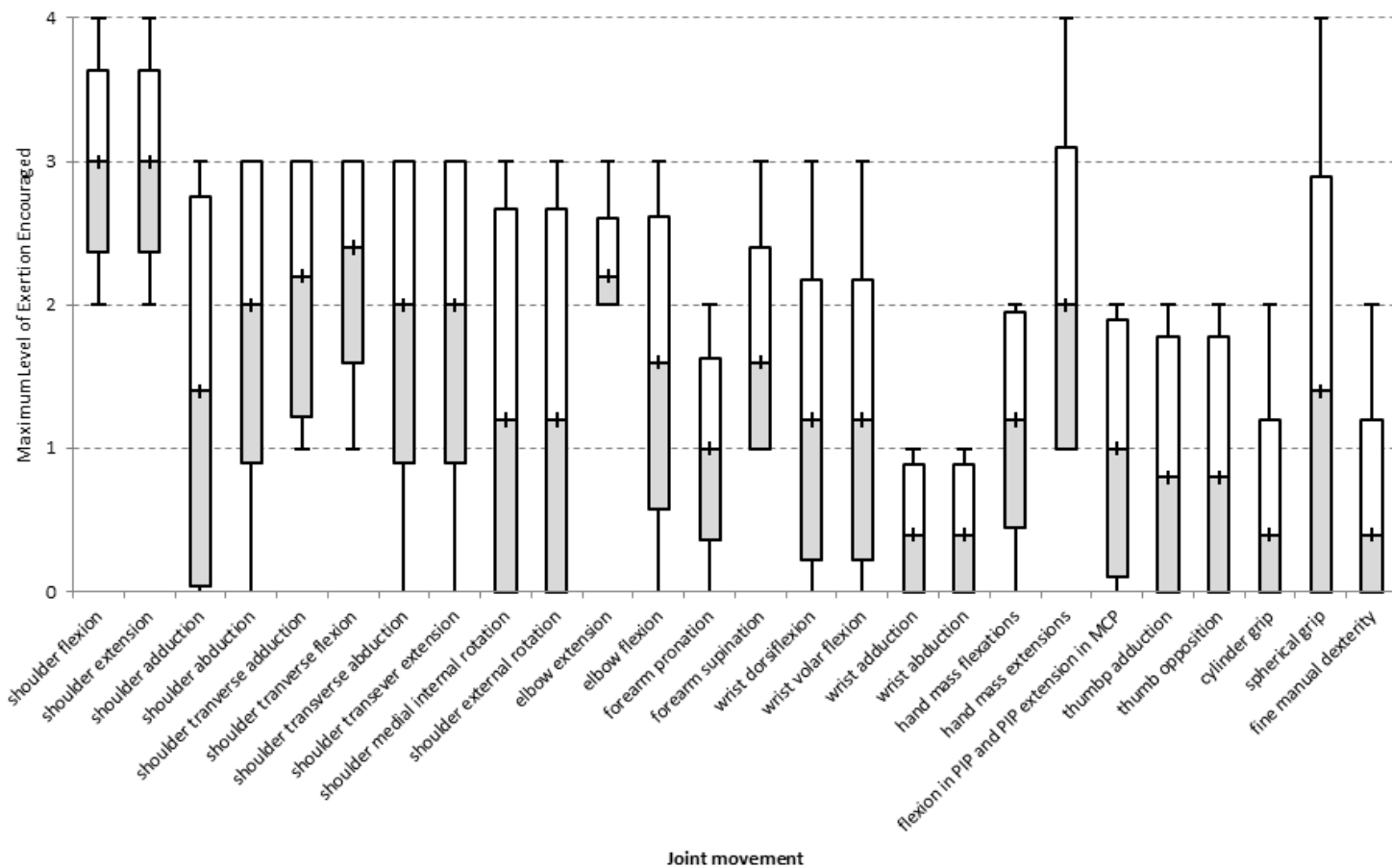
0. No motion required. 1. Reduced range of motion. 2. Partial range of motion. 3. Partial range of motion against gravity. 4. Full range of motion against gravity.

Figure 3. Minimum joint functionality required to play table tennis.



0. Not encouraged. 1. Encouraged but not required. 2. Implicit to fulfilling required task. 3. Explicit to fulfilling required task.

Figure 4. Joint exercise encouraged for bowling.



0. No exertion. 1. Low. 2. Medium. 3. High. 4. Dangerous

Figure 5. Maximum level of exertion encouraged for bowling.

DISCUSSION

The off-the-shelf Kinect game Kinect Sports was evaluated to assess the feasibility of using it in its current form as a stroke rehabilitation aid. Two games from the title Kinect Sports were evaluated – bowling and table tennis. It was found that both titles were highly exertive on the shoulders and arms, while no wrist, hand or digit functionality was required at all. Although it was felt that wrist and hand movements were encouraged through the context of the exercises, as the Kinect does not track these motions it cannot be guaranteed that the user is performing them during play. Due to the high level of arm functionality required, it is likely that the games would only be useable by stroke survivors who have recovered a significant amount of motor functionality. Qualitative information was also gathered from therapists during the study, who believed the exercises combined with the requirement to stand while playing presented a real danger of fall and injury during play. It is concluded that off-the-shelf Kinect games are not suitable for most stroke survivors for unsupervised rehabilitation.

Adaptations that could be made to the evaluated games would be to allow play from a sitting position to reduce the risk of a fall and further injury. The Kinect Sports game does not support seated play but a small number of Kinect COTS games do allow this, e.g., Fruit Ninja Kinect and Kinectimals. Our results also showed that games require a high degree of joint functionality to play especially with regards to shoulder movements. This limits the potential audience to stroke survivors who have already regained a high degree of joint functionality. This is because the games are designed for able bodied users without accessibility in mind. By altering the game design to rely on less exerting movements would increase the number of users who are able to play. One instance of this is in Kinect Sports Bowling where the player is required to hold their arm out to the side to pick up a ball. This is a difficult move and not possible for a large number of stroke survivors especially during rehabilitation. Providing alternative ways to perform these actions would allow people with less arm functionality to participate more fully.

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Chapter 100

LEAP MOTION CONTROLLER AND OCULUS RIFT VIRTUAL REALITY HEADSET FOR UPPER ARM STROKE REHABILITATION

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ABSTRACT

Intensive rehabilitation is important for stroke survivors but difficult to achieve due to limited access to rehabilitation therapy. We present a virtual reality rehabilitation system, Target Acquiring Exercise (TAGER), designed to supplement center-based rehabilitation therapy by providing engaging and personalized exercises. TAGER uses natural user interface devices, namely the Microsoft Kinect, Leap Motion and Myo armband, to track upper arm and body motion. Linear regression was applied to 3D user motion data using four popular forms of Fitts's law and each approach evaluated. While all four forms of Fitt's Law produced similar results and could model users adequately, it may be argued that a 3D tailored form provided the best fit. However, we propose that Fitts's Law may be more suitable as the basis of a more complex model to profile user performance. Evaluated by healthy users TAGER proved robust, with valuable lessons learned to inform future design. The majority of users enjoyed using the Leap Motion controller and VR Headset, the inclusion of visual cues shows a general improvement in target acquiring performance and that Fitts's Law can be used to linearly model user reaching and pointing movements in 3D environments. However, in some situations this is not the case, emphasizing the importance of user profiling to examine the user's kinematic behavior more intricately.

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INTRODUCTION

Upper limb rehabilitation following a brain injury, stroke, or other condition affecting upper limb movement, is carried out by occupational therapists and physiotherapists to recover and adapt the patient's movement and improve activities of daily living (ADL). Research confirms that rehabilitation is capable of improving arm function during both the early and late phases of stroke. However, effective therapy must be intense and requires the repetitive practice of task-related movements and actions (1). Repetitive task training involves intensive repeated practice of appropriate functional tasks and is thought to reduce muscle weakness and form a physiological basis of motor learning (2).

Virtual reality (VR) has significant potential to support self-management of such rehabilitation, in that it allows individuals to interact and train within stimulating, realistic virtual environments. It provides users with the opportunity to practice intensive repetition of meaningful task-related activities necessary for effective rehabilitation (3). A recent Cochrane review of 12 trials involving 397 participants for the upper limb, stated that the use of VR and interactive video gaming might be beneficial in improving upper limb function and ADLs; when used as an adjunct to usual care or when compared with the same dose of conventional therapy (4). This Cochrane review concluded that it is unclear at present which characteristics of VR are most important. However, they emphasized the need for pilot studies assessing usability and validity as part of the development process if designing new VR programs for rehabilitation purposes; these studies may also afford insight on the key VR characteristics for retraining of movement e.g., in reach and pointing tasks.

In this chapter we expand on our latest research (5), presenting a multi-sensory VR system, Target Acquiring Exercise (TAGER), and evaluate its usability and performance for upper limb rehabilitation. We evaluate Fitts's law as the basis of an adaptive system to model movement performance for reach and touch tasks in a 3D virtual space. TAGER evolved from previous VR and augmented reality (AR) testbeds developed by our research group (6) and particularly from initial research undertaken with the Leap Motion controller (7). TAGER utilizes several natural user interface (NUI) devices to track the user, namely the Leap Motion, Microsoft Kinect V2, and the Myo armband, and affords the option of wearing a VR headset (in this experiment the Oculus Rift DK1). In the experiment outlined in this paper, the tasks comprise basic 3D reaching and pointing exercises so we can effectively evaluate the system and user model. The experiment reported here focused on testing with healthy users to help us evaluate the user interface ahead of planned experiments with impaired users, and has helped us refine the user profiling system. A participatory approach to system and experimental design was taken; engaging with physiotherapists and occupational therapists in local health trusts, namely the Regional Acquired Brain Injury Unit (Musgrave Hospital, Belfast), the Stroke Unit at the Royal Victoria Hospital (Belfast), and third sector agencies, e.g., Brain Injury Matters (Belfast).

A core interest in VR as a rehabilitation tool is due to its potential to provide an enjoyable experience. However, VR systems are flexible technologies supporting feedback, capability adaptation, high intensity, repetitive, functional exercises to encourage motor control and motor learning. There are an increasing number of studies mainly focused on using commercially available hardware devices to support upper limb rehabilitation (4). Recent cheaper, high-quality commercial technology, such as Leap Motion and the Myo, offer new

opportunities for effective, center and home based self-managed rehabilitation. When combined with other existing technology, they have the potential to improve accuracy and reliability of performance monitoring, although research utilising these technologies is still limited. Feedback is of particular importance to patients, and VR systems have the potential to excel in providing rich, informative, personalized, just-in-time responsive feedback. Feedback cues in VR environments, when appropriately designed, can help users improve interaction performance. Rehabilitation systems have implemented multi-modal cues such as visual, tactile and auditory cues. The organization of movement is related to the quality of the viewing environment, particularly visual cues between the user's arm and the objects to improve spatial awareness (8). Tactile cues have also been reported to improve motor performance (9), and are mainly used to help users verify a successful interaction. Positive auditory cues provide user motivation to perform intensified repetitive tasks, represent temporal and spatial information very well and can improve motor learning (10).

Adaptation is an important usability factor within a VR rehabilitation user interface design, to enable a system to adapt both to a diversity of motor control capabilities and as rehabilitation progresses (6). This capability to adapt is important as a user may become frustrated if the tasks are too difficult or bored if tasks are too easy; thus maintaining engagement is vital in rehabilitation. Techniques proposed in the literature for adaptive VR rehabilitation systems include fuzzy logic and Fitts's Law (11). Fitts's Law is well known in the user interface community and has also been applied within the stroke rehabilitation (12) context. Fitts's Law models a user's motor skills by predicting the time to reach and touch a target based on a target's size (W) and the distance (D) from an origin (Eq. 1). The logarithmic element of the equation, known as the "Index of Difficulty" (ID), is used to quantify the difficulty of reaching a particular target. Thus, the movement time (MT) required to acquire a target is linearly dependent on ID , where smaller and further away objects are harder to attain.

$$MT = a + b \log_2 \left(2 \frac{D}{W} \right) \quad (1)$$

Equation 1 shows Fitts's original equation, but other researchers have devised variations of the equation to provide an improved model in different situations. Two popular adaptations of Fitts's Law are Shannon/McKenzie (eq. 2) and Welford (eq. 3), which were originally tailored to quantifying human movement behavior for 1D and 2D tasks. They have also been applied to 3D environments, but recently new forms of the equation have been devised to represent 3D interactive movements more accurately. Equation 4 (13) adapts Shannon/McKenzie (eq. 2) to include the addition of a movement direction parameter, to account for the consideration that MT is also dependent on the user's angle of motion (θ) on the Y axis from the origin to a target.

$$MT = a + b \log_2 \left(\frac{D}{W} + 1 \right) \quad (2)$$

$$MT = a + b \log_2 \left(\frac{D}{W} + 0.5 \right) \quad (3)$$

$$MT = a + b \log_2 \left(\frac{D}{W} + 1 \right) + c \sin \theta \quad (4)$$

OUR STUDY

The research described in this chapter has an exploratory emphasis and focuses on investigating VR NUI design, particularly (i) the reliability of tracking systems and the modelling of user motion via Fitts's Law and its variants, (ii) the aesthetic design of multi-modal cues, and (iii) the usability and acceptability of the VR headset. The purpose of conducting this research – and the subsequent follow up with impaired users – is to ensure that the underlying interactive interface and adaptive motion tracking system is as robust as possible before adding further user interface elements, game components, and connected health systems. Ultimately our intention is that the system will be robust enough for unsupervised usage by stroke patients.

Participant Recruitment

Healthy participants were enrolled in the experiment from students and staff at Ulster University. Inclusion criteria included that participants should be 18 years old and over, have no vision issues (e.g., blurred vision, color distortion, light sensitivity, depth perception), nor any disability that affected the upper extremity. Participants completed a questionnaire to gather information regarding IT and gaming literacy and to determine inclusion in the experiment.

Target Acquiring Exercise (TAGER)

TAGER is a custom designed 3D pointing exercise for upper limb rehabilitation, designed based on requirements from research (14) and clinician involvement. TAGER utilizes a number of technologies that work together to monitor and provide feedback to users while completing upper arm rehabilitation tasks. The Leap Motion controller is a small desktop NUI which contains an infrared camera specifically design to track fingers, hands and arms. It tracks up to 20 bones per hand, with 150° field of view and approximately 8ft³ of 3D interactive space. TAGER uses the Leap Motion as the main interactive device within the virtual world for tracking motion and facilitating target acquisition. Microsoft's Kinect V2 is similar to the Leap Motion, though instead of sensing hands it senses motion of the human skeleton. We collect data of all joints in the upper body in motion with the goal in a future version of providing guidance on suitable functional task movements and other factors. It is natural for a person with an affected limb to move their whole body forward rather than extending their arm, especially if tired. As this would hinder improvement through the rehabilitation process, system feedback and guidance can be crucial. The Oculus Rift DK1 (VR Headset) contains a 7" screen with a resolution of 1280×800 (16:10 aspect ratio) and a 90° field of view and enables head positional and rotational tracking. User head motion controls the viewpoint within a virtual world. The Oculus is investigated in this experiment to determine if it is acceptable for use and could potentially be used to increase spatial awareness. The Myo armband slides onto a forearm, and uses electromyography (EMG) technology with eight medical grade EMG nodes attached, that read electrical signals from

the muscles. The Myo armband also includes an accelerometer and gyroscope to track movement and orientation on the forearm. The use of the Myo armband here is to collect data to be stored for future studies helping identify changes in muscles, which could highlight factors such as fatigue and correctness of exercise. The Myo armband also includes tactile feedback capability, for example, vibrations on the skin, which are used to introduce tactile cues into the system.

Experimental Setup

The experimental process comprised three stages: (i) a training stage which gives the participant ten minutes to familiarize themselves with the technology and practice interaction. The training tasks are very similar to the real trials. Through system testing and observing users before the actual experiment it was decided that ten minutes training would be sufficient time for the participant to familiarize themselves but not cause fatigue. After training, the patients are given a short two minutes' rest period before the next stage (ii), which is the complete TAGER trial. In stage 3, after each user completes the trial, a short discussion takes place gathering any comments the participant might have and for the investigator to ask questions related to the user's experience of the system. Throughout the experiment, the participant was closely monitored by the investigator who provided assistance if any problems arose. The experiment was strictly controlled; the location of each trial was always the same as was the equipment used. Equipment arrangement was identical for each patient ensuring no other possible variables could affect the collected results. Figure 1 illustrates the layout of the environment.

TAGER's 3D virtual environment is the inside of a basic walled room (no wall at the front) with a large start button on its floor. The user was prompted to push the button with their virtual hand to begin. An icosahedron shape was purposely chosen as the target object for its geometric properties, especially since visual cues are required to enhance spatial awareness. With changes in viewing angles, objects with a greater number of faces and edges such as icosahedrons, provide more clarity of visual cues (15). Each repetition (4 per level) contains 27 icosahedrons to target, all at different locations, a total of 108 per level (4×27). When the start button was pushed a single icosahedron appears randomly at any of the 27 locations. The user moves their virtual hand around the 3D environment touching each icosahedron that appears. When touched, the icosahedron disappeared, and another icosahedron appears on the floor of the room at the location of the start button. We call this object the origin; this approach was used to provide consistent movement trajectories. All objects were intentionally placed at fixed locations and at fixed distances from the user's view to simplify analysis.

The TAGER VR software for this research was constructed with ten levels; the first and last levels were identical enabling us to compare performance over the period of the session – considering learning and fatigue effects. All other levels were unique concerning scene attributes such as target object scale, multi-modal cues and VR headset use; to investigate the impact they had on user movement behavior (see Figure 2). Levels 2-9 were randomized per user to eliminate potential bias in the ordering; a rest period provided between each level and repetition. The unique levels comprise different combinations of scene attributes such as shadowing and proximity color change for visual cueing. Tactile cues were included using the

Myo Armband where a vibration was sent to the user’s arm upon successful target acquisition. We also changed the scale of the objects; objects were sized accordingly as 2, 3.5 and 5cm. Objects were scaled to discover the impact it has on cues. For example, larger objects were expected to give greater clarity to visual cues and thus quicker arm kinematics. These scene attributes helped build knowledge on the impact they have on arm kinematics, spatial awareness, movement speed and accuracy.

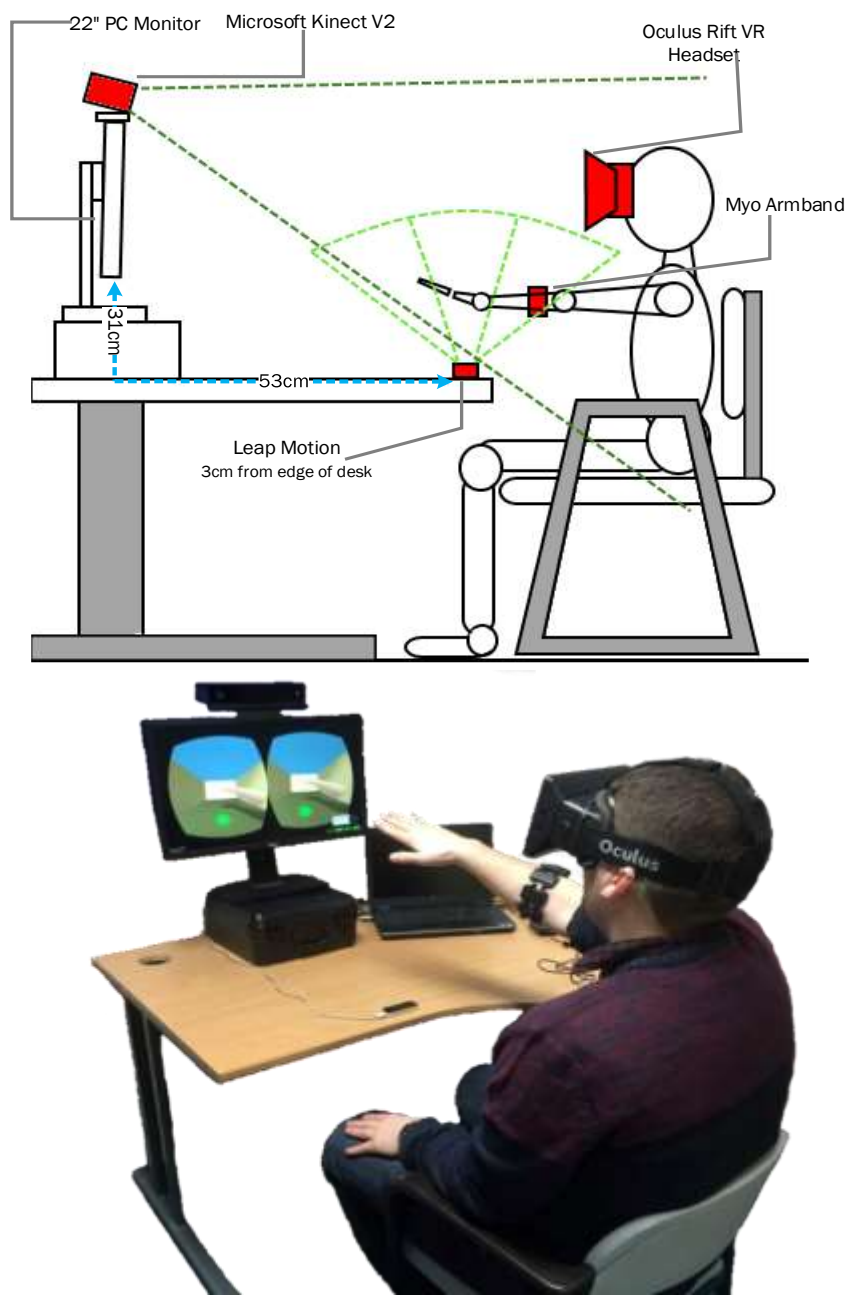


Figure 1. The Experiment setup showing the equipment locations and typical user interactions.

RESULTS

The above experiment was undertaken to investigate core aspects of TAGER. Data was recorded every tenth of a second from all input devices, and *MT* calculated and recorded to file automatically from tasks for analysis of the movement models. Of the 26 participants, three were excluded from data analysis due to missing data or system issues (loss of tracking).

Usability

Of the 26 participants, 77% reported that their experience using the VR headset was enjoyable, no motion sickness or other health-related effects were reported. 43% of people commented that they perceived their performance to have improved while wearing the headset, while 50% said that they needed time to adjust when first wearing the headset. We compared user performance between the VR headset and monitor use, with and without cues. We used a paired t-test to determine significant differences between users' average *MT* and found no significant difference between Cues ($T=1.681$; $DOF=21$; $p=0.053$) or No Cues ($T=1.591$; $DOF=21$; $p=0.063$). Though, there was a consistently slower user response when using the headset (Table 1). In other words, the participant's subjective experience was at odds with the objective measurement. Although this is not a significant issue so long as the effect is consistent among users, it is a consideration for future interaction design. Discussion with health professionals reinforced the importance of aesthetic cues in rehabilitation VR system design. We provided feedback on target proximity and acquisition through lighting and shading, as well as vibration from the Myo armband. Figure 3 shows variation in interaction difficulty with cues (C) or without cues (NC) for different sized objects (large – LRG, medium – MED and small – SML). While results were generally as expected – i.e., cues supported improved efficiency in target acquisition. It was not clear that cues improve performance for MED cue acquisition and more investigation is required. More detail will be covered on the effect of cues in the next sub-section.

**Table 1. The mean movement time comparing VR headset and PC monitor:
Cues(C), No cues (NC)**

Exercise	VR (NC)	Monitor (NC)	VR (C)	Monitor (C)
Mean MT (ms)	1174.2	1107.4	1165.0	1115.4
T-test P=(0.05)	0.063		0.053	

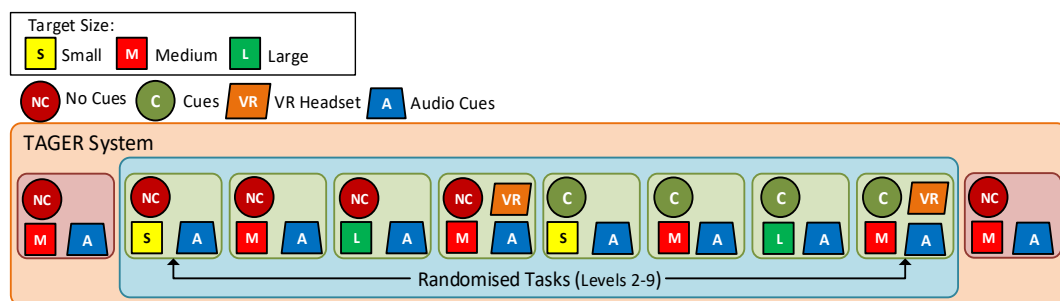


Figure 2. TAGER's level layout and scene attributes.

An objective way to evaluate system feature effectiveness in TAGER is to record target acquisitions (hits) against movement times. A target has been successfully acquired when the user's hand collides with the surface of the target object without leaving the Leap Motion's detection area. Hits are classified as unsuccessful when the user's hand leaves the boundaries of the Leap Motion's detection area before hitting the target; users were required to return to within the boundaries to hit the target. Users could not progress until they acquired the current target, even after registering an unsuccessful hit. It was found that targets positioned at the center depth (relative to the user) were more successfully acquired – a mean of 29 successful hits per task over all participants (Front = 22, Back = 26). Closer objects appear to have been harder to attain, suggesting we may need to consider moving the minimum distance further away (along the z-axis) or move objects closer to the z-axis along the x and y-axes. The latter suggests a cone area (16) for object placement – with the cone pointing towards the user – rather than a cuboid (which maps well to the Leap Motion's detection area). This approach might account better for arm kinematic differences in attaining targets in close and far locations. Users attained targets in all sectors with reasonable success, though on average there were 31 (28%) unsuccessful acquisitions per level from a total of 108 targets. This may be considered to be quite high for healthy users; if the Leap Motion was mounted on the VR headset, changing position so that the leap camera is pointing forward (user's facing direction) could possibly provide a more natural interactive space, and reduce unsuccessful acquisitions. TAGER's experimental design implemented two identical levels one at the beginning and end of the experiment, enabling investigation of the potential learning effects indicated by improved performance or user fatigue resulting in performance decline. The mean completion times for all users for the start task was 1257.8ms while for end tasks the mean completion time was 1081.4ms suggesting that, despite having 10 minutes training time at the beginning, user performance significantly improved over the experiment ($T=4.60$, $DOF=21$, $p=7.7E-05$). There are also some indications of fatigue (or loss of concentration) among several users. Analysis of experimental data in the next section enabled us to investigate the intricacy of user profiles.

Model Effectiveness

As outlined earlier we recorded user motion data in reaching from an origin to touch an object target at various distances. Figure 4 shows the lines fitted to the data through regression for four well-known forms of Fitts's Law.

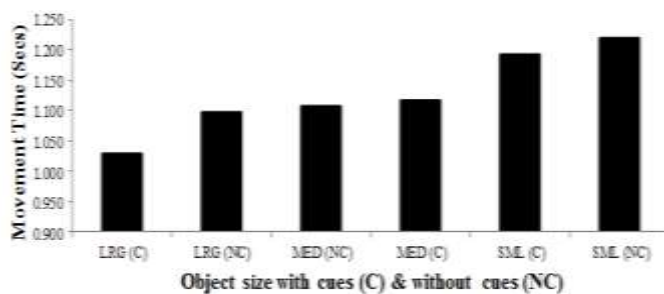


Figure 3. The variation of interaction difficulty arranged by the magnitude of the mean movement time.

Table 2. The mean y-intercepts across each TAGER level for all forms of Fitts Law

EQ	SML (NC) (ms)	SML (C)	MED (NC)	MED (C)	LRG (NC)	LRG (C)	VR (NC)	VR (C)	Start	End	Overall Mean
(1)	189.3	77.4	270.7	161.6	392.7	343.4	70.6	-41.6	254.1	356.7	207.5 ± 89.3
(2)	-25.3	-152.2	95.9	-30.8	254.4	209.2	-107.9	-293.5	49.6	213.2	21.3 ± 110.9
(3)	86.4	-37.9	121.5	-7.5	210.2	166.6	-122.8	-250.8	74.7	224.4	46.5 ± 107.0
(4)	319.3	203.3	279.9	243.4	357.7	346.6	203.8	185.0	288.3	374.2	280.2 ± 83.6

Table 3. The mean slopes across each TAGER level for all forms of Fitts Law

EQ	SML (NC) (ms)	SML (C)	MED (NC)	MED (C)	LRG (NC)	LRG (C)	VR (NC)	VR (C)	Start	End	Overall Mean	Bits Per Sec
(1)	339.3	370.1	355.8	406.2	366.9	356.2	463.7	510.8	427.3	308.4	390.5 ± 46.0	2.5
(2)	315.7	343.2	316.6	358.4	311.0	301.9	392.7	454.3	378.1	271.6	344.4 ± 41.1	2.9
(3)	362.8	396.7	396.6	451.2	421.8	409.4	515.8	566.6	475.7	344.6	434.1 ± 51.2	2.3
(4)	247.7	273.1	275.1	290.5	282.1	261.7	318.5	324.1	322.9	236.2	283.2 ± 32.7	3.5

Tables 2 and 3 show the average regression values of the y-intercept and slope values for all participants. Murata's 3D equation (4) provides y-intercept values ranging from 196 to 363, which are more representative of human movement response (typically 200 to 300ms); it also provided a more gradual gradient giving a more appropriate model of *MT* against *ID* (17). Thus, while all four equations provided similar results, we focused on Murata for further analysis. Figure 5 shows typical results while wearing the VR Headset with and without cues for medium sized targets and Murata's 3D form of Fitts's law. In Figure 5 when cues are included, user 1023 seems to improve her performance shown by a more gradual slope (decrease in value), the y-intercept (reaction time) towards a better representation of the typical human reaction time values and R^2 correlations are very similar. This experiment was fundamentally exploratory in nature. We used Fitts's law and linear regression to develop a user model, which will help us understand how best to create an adaptive system that can personalize interactive tasks. Table 4 provides user profiles from a proposed model comprising parameters such as regression line data, statistics on the residuals of the line fit, task performance and user information. Considering the explicit performance metrics (hits, movement times), target hits provided an informative statistic, and capable users are readily distinguishable from less able. Only one person had a score of less than 500 /1080 with the lowest score 472. The highest score was 900, and the average score was 754.74. Mean successful hits per person rose from 73.91 at the start to 77.22 at the end from a total of 108. The mean percentage improvement in hits over the experiment was 6.92% illustrating diversity of learning and fatigue effects among participants. The mean percentage reduction in *MT* was 13.79%. The average change in line fit according to R^2 and associated values were not significant (due to the spread of data values there are several valid lines). Change in hits between the start and end tasks in the experiment were potentially revealing of a possible learning effect (improvement) or mental/physical fatigue. A learning effect provided a challenge to adapting task difficulty per user – it is preferable to adapt difficulty based on Fitts's law only after the learning effect stabilizes. Nonetheless, it was helpful to identify that a user remains in a learning phase so that additional support can be provided. However, *MT* and hits should not be considered independently, as some users may slow down deliberately to improve hit performance. The mean slope gradient decreased by 26.85% and the mean intercept decreased by 29.82%. Shallower slopes indicated a more skilled response as there is less difference between acquiring close and far objects, while a rise in the intercept value moves the intercept closer to physiological reflex norms (generally between 200 and 300ms). Additional user profile information can be gained from the residual statistics of regression lines and the changes between start and end tasks. Mean standard deviation dropped by 19.28% and mean variance also fell by 32.49%, suggesting that on average users were becoming more accurate. Range dropped by 10%, supporting this proposal. Kurtosis of residuals increased by over six times the magnitude (positive values) suggesting an increased number of data points with a small deviation from the regression line. On the other hand, skew also increased 44.4% (also positive), which after examining the regression graphs of users we believe was due to users overshooting the target position and having to change direction. From our results, we did not identify a strong relationship between performance and user age or gender.

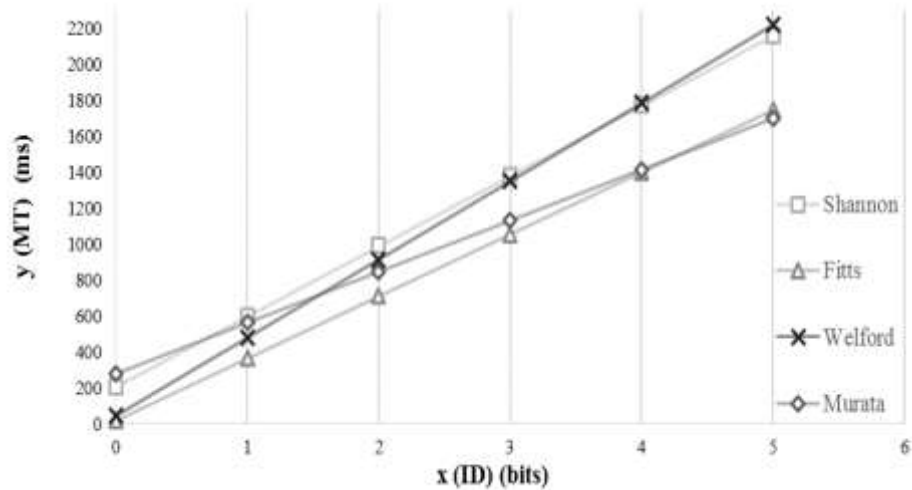


Figure 4. Regression line from our data for the four popular variants of Fitts Law.

Table 4. Participant statistics calculated over time including residual statistics, regression analysis and performance data to define a user performance profile

User	1001	1002	1009	1013	1019	1023	1026
Age : Gender	20 : F	20 : F	42 : M	24 : M	66 : F	54 : F	24 : F
Start (Residual)							
Standard Deviation	0.354	0.461	0.257	0.368	0.313	0.408	0.378
Kurtosis	0.085	-1.141	0.031	-0.238	-0.331	-0.262	0.376
Skewness	0.986	0.394	0.996	0.286	0.394	0.785	0.980
End (Residual)							
Standard Deviation	0.111	0.275	0.194	0.242	0.420	0.285	0.235
Kurtosis	5.852	0.985	2.540	-0.627	1.927	1.197	0.498
Skewness	1.834	1.227	1.515	0.187	0.952	1.143	1.289
Start Regression							
R ²	0.115	0.076	0.228	0.453	0.343	0.112	0.189
P-Value	4.05E-03	9.28E-02	8.60E-07	1.78E-08	1.79E-08	1.17E-03	3.92E-05
Slope	0.268	0.312	0.273	0.602	0.517	0.348	0.388
Intercept	0.352	0.360	0.268	-0.289	-0.212	0.475	-0.121
End Regression							
R ²	0.266	0.024	0.348	0.446	0.224	0.392	0.004
P-Value	3.80E-05	2.54E-01	2.83E-09	7.42E-09	3.18E-06	2.44E-11	5.77E-01
Slope	0.133	-0.109	0.293	0.445	0.481	0.509	0.033
Intercept	0.359	1.220	0.092	-0.070	0.116	-0.216	0.703
Performance							
Targets Hit (1080)	794	554	848	638	828	900	788
Start Hits	70	38	96	55	78	91	83
End Hits	73	57	85	59	88	92	74
% Change Hits	4.29	50.00	-11.46	7.27	12.82	1.10	-10.84
Start Mean Time	1.167	1.292	1.063	1.522	1.391	1.497	1.055
End Mean Time	0.768	0.884	0.952	1.270	1.580	1.262	0.802
% Change Mean Time	-34.19	-31.59	-10.42	-16.54	13.64	-15.66	-23.96

Data from seven of the twenty-three participants provided a poor fit to the model based on start task motion data. Data after the end session from five participants had a poor regression fit, though two of these participants were different from the seven identified from the start data. These two people may have been overly tired or bored, rather than being less capable, highlighting the need for intricacy and careful analysis of the profiles. To consider this further it was necessary to examine a few representative individual user profiles (Table 4). User 1026 was an informative example, who started well with a strong profile but whose hits and mean MT declined significantly during the end task. Despite having residual statistics during the end task that show less variability (indicating more effective target acquisition for a significant number of targets), hit score actually decreased, while skew and kurtosis both increased (suggesting more overshooting of the target), and R^2 and the associated P-value suggested an unreliable regression fit (possibly due to an increase in outliers). 1026's mean MT dropped by a 23.96%, which suggested, considering the user profile, that during the end task this user may have adopted a high-risk strategy. This is in contrast to 1019, who appeared to have become more conservative, and by moving slower (13.64% increase in mean MT) improved their hit score by 12.82%. This considered approach by user 1019 and other participants seemed to improve the likelihood that the user motion data corresponds to Fitts's law (and variants). User 1002 had the weakest start having the lowest number of hits in the initial task but improved hit performance by 50% and mean MT by 31.59%. However, even by the end task, this user's motion could not be applied to Fitts's law reliably, and they had less than a 50% hit success rate in the final task. User 1023 exhibited arguably the most sustained success, having the highest overall hit success and start and end scores of 90 and 91 respectively and overall an improved profile (based on regression values). As with user 1019, 1023 was not particularly fast but improved speed during the experiment. Three participants got slower over the experiment and all of these achieved higher scores in doing so. Five participants got lower hit scores and all of these, but one had much less adequate regression fit and three of these exhibited faster mean MTs. Further investigation is required to understand whether users with these profiles exhibit a decline of interest – the tasks were repetitive and several of these participants were quite skilled at the tasks – or due to mental/physical fatigue.

DISCUSSION

In this paper, we presented an initial version of the TAGER VR upper arm stroke rehabilitation system, which utilizes several inexpensive, commercially available input devices and a VR headset. We reported on an experiment that focused on the usability of the system and the use of various forms of Fitts's law to linearly model user motion - movement time against an index of difficulty – for reach and touch tasks in a 3D environment using a Leap Motion controller as an input device. Most users enjoyed using the Leap Motion device and perceived tasks to be easier with the VR headset on. No motion sickness or other negative health-related effects were reported. The importance of cues has been reported widely in the literature, and while our results show a general improvement in object acquisition with visual cues, we found the impact on targeting medium sized objects to be unclear. We outlined results from fitting user motion data to four forms of Fitts's law with each of the equations

exhibiting a similar success. Examining the statistics of the regression process based on Fitts's law we found that these equations could be used to linearly model user motion with the Leap Motion controller in a range of setups including the utilization of a VR headset. However, the data point variance across the regressed line was quite high, and Fitts's law should not be applied as a simple linear model of user motion equally to all users. We recommend that a form of Fitts's law, for example, Murata's 3D version could be used as part of an intelligent system to profile users. Although the motion of most users could be modelled effectively using Fitts's law, we found a few users who found the NUI so difficult that they could not be modelled linearly. Most of these users required more training than we expected but in some cases, users appeared to become tired or bored. A more complex profiling system will help to identify training requirements and distinguish between loss of interest and mental or physical fatigue. We intend to develop the profiling system with further experiments and subsequently investigate the system with impaired users.

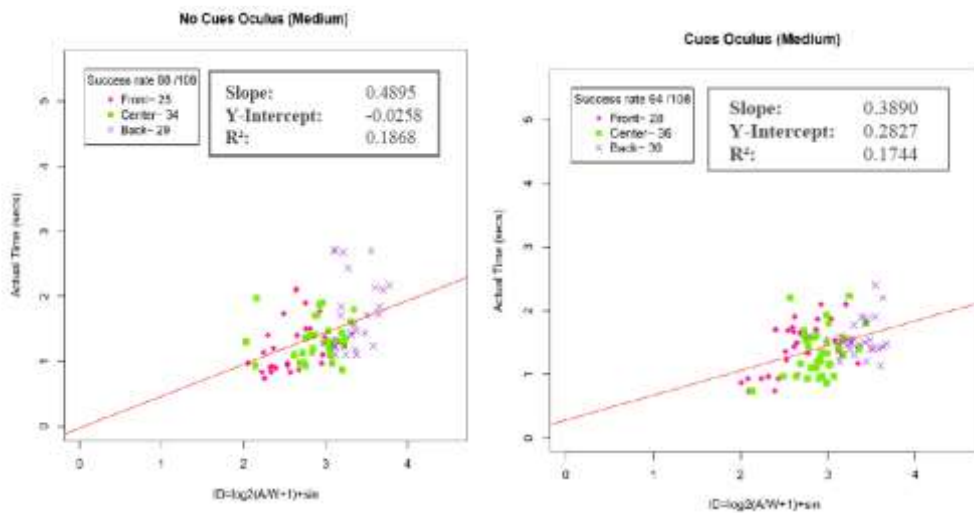


Figure 5. Murata's 3D model of movement for user 1023 while wearing the VR headset, plotting Index of Difficulty against the user's response time to successfully target medium sized objects. Depth (z-axis) is distinguished by 3 locations in the virtual world relative to the user: front (red diamonds), center (green squares), and back (purple crosses).

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Chapter 101

HOME BASED VIRTUAL REHABILITATION FOR UPPER EXTREMITY FUNCTIONAL RECOVERY POST-STROKE

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ABSTRACT

After stroke, sustained hand rehabilitation training is required for continuous improvement and maintenance of distal function. An ideal home-based telerehabilitation system has to be low cost, easy to set up, effective in motivating the user to use it every day, generate progress reports to the user for self-tracking, and provide daily monitoring to remote clinicians. In this chapter, we present a system designed and implemented in our lab: the NJIT Home-based Virtual Rehabilitation System (NJIT HoVRS). A single subject proof of concept study was conducted and demonstrated that this system is easy to access and effective in motivating subjects to train at home.

INTRODUCTION

Stroke remains the leading cause of serious, long-term disability in the United States, with over 6.8 million stroke survivors (1). Although the incidence of death from stroke has been decreasing due to new medical treatments during the acute episode, this still leaves a significant number of individuals permanently disabled. Only 10% of survivors recover

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completely, the majority have a long-term or lifelong need for help to perform activities of daily living and require further rehabilitation (2) as a result of a significant brain injury. Deficits in motor control affect the stroke survivors' capacity for independent living and economic self-sufficiency. The impact of even mild to moderate deficits in hand control in particular, affect many activities of daily living.

Intensity and progression have been proposed as key factors in successful stroke rehabilitation. A very recent article, offering a theoretical framework with which to develop future post-stroke clinical trials, proposed that intensity and progression are the active ingredients of a rehabilitation program that drive neural plasticity and lead to positive functional outcomes (3). Studies have shown that sustained hand rehabilitation training is important for continuous improvement and maintenance of function (4, 5). If the amount of therapy is critical to rehabilitation, our current institutional limitations undermine the probabilities for successful outcomes. Time constraints and expensive personnel as well as the restricted length of stay in both acute care hospitals and rehabilitation restrain the provision of an adequate dose of training for persons with stroke. After discharge from the inpatient stay, access to rehabilitation therapy can be difficult for some patients. This is due in part to inadequate insurance, lack of transportation, and the patient's dependence on their caregiver. Having access to long-term rehabilitation training anywhere and at any time is necessary for sub-acute and chronic patients to continuously improve their functional abilities. There is a momentum building for increased attention to the development of technological interventions intended to support repetitive on-going home-based practice for better recovery of upper extremity motor function.

Innovative telerehabilitation systems have been developed using information and communication technologies to provide rehabilitation services at a distance. Many studies have developed video-game driven systems from commercially available gaming consoles such as Wii and Microsoft Kinect (6). Other groups have examined the use of custom-made tele-rehabilitation systems (7, 8). Most of these systems target arm or postural control, and require more space than may be available in the patient's home. These systems do not address hand rehabilitation. There is a vital need to explore intensive home-based upper extremity interventions that focus on the hand. An ideal home-based telerehabilitation system has to be low cost, easy to setup, able to motivate the user to use it every day, generate progress reports to the user for self-tracking, and provide daily monitoring to remote clinicians. Exciting new technologies have now made this approach possible and hold promise for long-term benefit. These technological advances - for the first time - allow for virtual reality simulations interfaced with discrete finger and hand tracking that is affordable and easy to use.

The NJIT Home-based Virtual Rehabilitation System (NJIT HoVRS) integrates the Leap Motion Controller and virtual reality technology to provide focused rehabilitation of wrist, hand, and finger movement. This paper presents the design concepts behind the NJIT HoVRS and the results of a proof of concept test. We believe that the NJIT HoVRS is a powerful tool that will give patients access to affordable, effective, and long-term rehabilitation in their home, allowing them to maximize and sustain stroke recovery, while increasing their overall quality of life.

SYSTEM DESIGN

NJIT HoVRS has two sub-systems to deliver home-based training: 1) a patient based platform to provide the training and 2) a server based online data logging and reporting system. In the patient's home, a cross platform virtual reality training application runs video games (developed in the Unity 3D game engine using the language C#) on their home computer. The Leap Motion Controller (LMC) infrared tracking device is used to capture motion of the hand and arm movement without requiring wearable sensors that may be difficult to put on independently or could potentially restrain movement.

Hardware

The Leap Motion Controller (LMC) (see Figure 1) is a \$50 computer hardware sensor that captures detailed hand movement as well as hand gestures. The heart of the device consists of two cameras and three infrared LEDs. A data validation study showed the LMC to be accurate and reliable as long as the target is within its visual area (± 250 mm of the LCM center) (9). The device's USB controller reads the sensor data into its own local memory and performs any necessary resolution adjustments. This data is then streamed via USB to the Leap Motion image API. From there, we programmed the system to feed tracking data into virtual reality activities by calling the Leap Motion API.

If the patient's arm is severely impaired and he/she cannot support the hand against gravity above the Leap Motion Controller, the Armon™ Edero, a spring compensation system, can be provided to the subject (see Figure 1). The Edero provides 12 different levels of passive support allowing it to accommodate a wide range of patient sizes and strength levels. It requires a single, setting that can be provided during the patient's initial evaluation. Minimal instruction is required to teach patients to readjust support levels on their own



Figure 1. Left: RAVR-Home architecture design. Upper right: Leap Motion Controller. Lower Right: Armon Edero™ arm support.



Figure 2. Left: RAVR-Home architecture design. Upper right: Leap Motion Controller. Lower Right: Armon Edero™ arm support. Figure 2. a: Maze, b. Whack-A-Mole, c. Soccer Goalie, d. Bowling, e. Fruit Picking, f. Fruit Catching, g. Car game, h. Hand Fly, i. Wrist Fly, j. Arm Fly, k. Piano, l. Breakout.

Software

A user-friendly GUI interface lists all of the training activities allowing patients to choose which activity they want to begin with using just one mouse click.

Currently twelve games have been developed, each one designed to focus on training a specific hand or arm movement such as wrist rotation or finger individuation (see Figure 2). An avatar is at the corner of all game environments to provide verbal praise and encouragement as therapists usually do in real world. Therapists configuration page can be enabled by pressing “T” for game condition pre-setup, such as work space size, game challenge level, etc (see Figure 3a). All games are downloadable via HoVRS website.

Maze: Player controls the movement of the virtual character through their hand location. The score is defined by the number of spheres the character intercepts along the maze path. The character falls if he deviates from the defined path. As the game level increases in difficulty; the platforms and bridges that the character is guided over become narrower and sharp turns become more frequent.

Whack-a-mole: The player controls the position of the hammer through arm movement in the horizontal plane. Rotation of the hammer is controlled by pronation/supination. Success score is defined by the amount of moles hit.

- Soccer goalie:** The player controls the position of the virtual hand to block the approaching soccer balls from hitting the goal.
- Bowling:** The player reaches forward and extends their fingers to apply force to the ball to knock over the pins.
- Fruit picking:** The player must reach and use a pinch movement with their thumb and forefinger to pick apples and oranges from trees and sort them into the correct basket to increase the score.
- Fruit catching:** To catch the fruit, the player controls location of a collection basket through horizontal arm movement. To increase the score, the player uses forearm pronation/supination to drop the collected fruit into a second basket. The frequency of falling fruit decreases when the player misses 25% of the falling fruit
- Car game:** The player practices opening and closing their hand to control the car speed. Closing the hand slows the car down allowing it to maneuver over speed bumps.
- Hand flying:** The plane speed is constant. The player controls vertical position of the plane by opening and closing their hand. Success score is defined by the number of floating spheres intercepted.
- Wrist flying:** The plane speed is constant. The player controls vertical position of the plane by changing the pitch of their hand at the wrist. Success score is defined by the number of floating spheres intercepted.
- Arm flying:** The plane speed is constant. The player controls the horizontal position and roll of the plane by moving their arm left and right and pronating/supinating their forearm. Success score is defined by the number of floating spheres intercepted.
- Piano:** The player plays a song by pressing the highlighted key with the indicated virtual finger. The amount of finger individuation required to successfully press any keys increases as the player progresses. However, it scales back down when they are unable to hit the key for 6 seconds.
- Breakout:** The paddle used to direct the ball follows the palm position of the player. The ball is directed by bouncing off the paddle and hitting the bricks above to clear the screen.



Figure 3. a. Therapist setup interface; b. Calibration interface; c. Hand not visible warning.

System Calibration and Hand Position Correction

A comprehensive calibration procedure (see Figure 3b) is used to measure each subject's active range of motion within the LMC visual area and this range is scaled to fit into the video game virtual environment. To create greater ease of use, games have been designed to display a red frame (see Figure 3c) to guide the subject back to the LMC visual area whenever his/her hand deviates from the calibrated range.

Data Collection

Performance and kinematic data collected during rehabilitation training using the NJIT HoVRS is securely transferred to Amazon Simple Storage Service (S3) at the end of the each training session for offline analysis. Patient data is automatically processed to produce daily progress reports. Therapists can securely access the reports through a password protected website, which will also host system updates.

SYSTEM VALIDATION

In order to evaluate NJIT HoVRS’ ease of use and reliability, a 45 year old female patient with moderate hemiparesis of the upper extremity (UEFMA = 45) due to a cortical stroke was recruited. She was a police detective prior to the stroke and was able to return to full time deskwork 6 months after the event. She lives alone but her family lives nearby and gave her a lot of support during her rehabilitation. She had little to no video game experience prior to this training. The system was placed in her home. Without help from the therapist or engineer, she successfully finished 7 thirty minutes training sessions utilizing all twelve of the games at least twice (Figure 4).

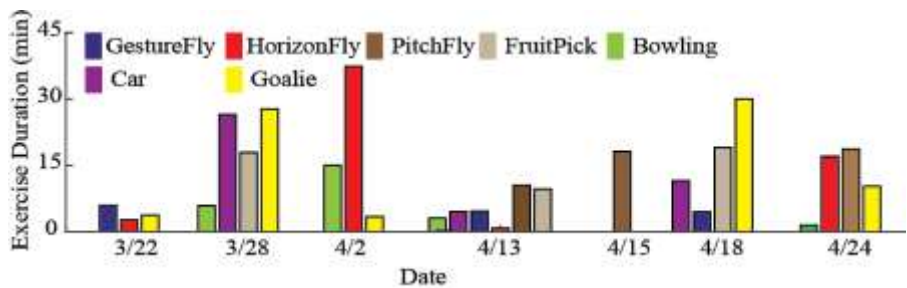


Figure 4. Exercise duration for each gaming activity over one month of therapy, as reported by the HoVRS website.

Car Simulation

As shown in Figure 5 (left panel), the subject was able to maintain her hand above the Leap Motion and control virtual car speed by opening and closing her hand. As the simulation went on, she gradually opened her hand more than she was capable of during the initial calibration.

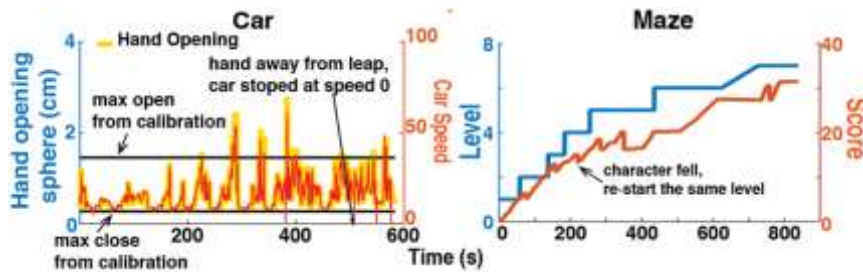


Figure 5. Performance during a single session of Car (Left) and Maze (Right) simulations. Left panel: Two horizontal lines indicate maximum hand closing and opening during initial calibration. Arrow indicates moment when hand tracking was lost and the car speed dropped to zero. Right panel: Arrow indicates moment when the avatar fell off the maze plane and the game restarted at the beginning of the level.3

Maze Simulation

In the maze simulation (Figure 5, right panel), after successfully reaching the target, the subject will advance to a higher level that requires more hand stability. If the subject fails to reach the target, the score collected from the current level will be deducted and the subject starts again from the beginning of the current level. As shown in Figure 5 right, our subject was able to successfully pass the first four easy levels. However, as difficulty level increased, the subject started to slow down and make multiple attempts to complete fifth level.

Questionnaire

In a post-training questionnaire, the subject noted the system's ease of use and a desire to incorporate the system as part of her on-going therapy. Subject felt that this therapy system was engaging. In her comments, she said that the system is easy to use, and she almost forgot that she was participating in rehabilitation training while she was focusing on trying to complete the computer tasks. Her arm was tired after doing the training, but not her hand. She felt this system would improve her hand motion. She wished it could have been part of her original therapy and wanted it to be part of her on-going therapy. Subject said most of the games were engaging. She was able to understand the requirements and learn how to play them quickly. The easiest game for her was the Car simulation because it had a one-dimensional control requiring only hand opening and closing to manipulate the car's speed. Her favorite game was the Arm Flying simulation because its soothing music made her relax and enjoy the training. The most difficult game for her was the piano because it required both arm stability and finger individuation. The most confusing game was Whack-A-Mole because different lighting and random appearance of the moles provided too much information and caused confusion about how to proceed. The most non-intuitive game was Soccer Goalie because the orientation of the virtual hand and the real hand did not match. The virtual hand displayed in the virtual environment was vertical with the palm facing forward, while the real hand was horizontal with the palm facing down.

New Jersey Institute of Technology's Home Based Virtual Rehabilitation System

CHOOSE PATIENT NAME AND GAME TO SEE PROGRESS

LK ☒ Bowling Submit

- BreakOut
- Cars
- Flying_gesture
- Flying_Pitch
- Flying_Yaw
- FruitPick
- FruitCatch
- Goalie
- Maze
- Plano
- WackAMole

Init	Day	Date	Game	Duration	MaxOpenWholeHand	MaxCloseWholeHand	ROM WholeHand	ROM ThumbPIP	ROM ThumbMPJ
LK	1	3222016	GestureFlying	398	413.1399616	186.0786919	227.0612697	24.80610692	0.308311581
LK	2	4132016	GestureFlying	313	415.1083938	183.1318084	231.9765854	17.10316018	0.000151885
LK	3	4182016	GestureFlying	300	430.6113351	190.6208496	239.9904854	30.12570069	1.192360472

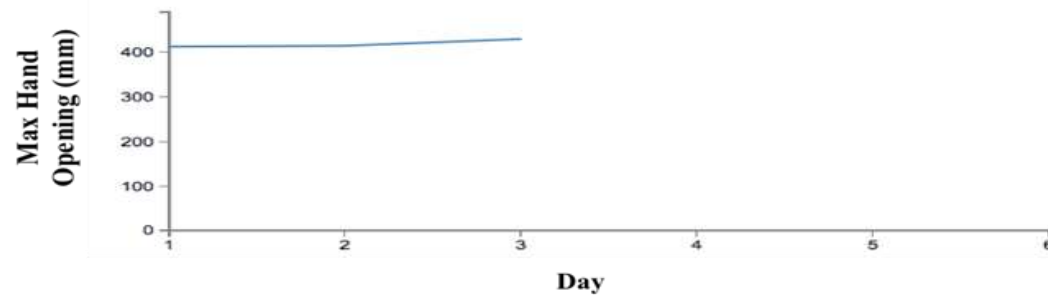


Figure 6. Therapist can monitor subject's daily progress via HoVRS website. Plot shows the sum of distances between the palm and five fingertips.

System Server for Data Monitoring and Visualization

All training data could be accessed remotely from the data server via HoVRS website. The therapist can access daily training data of a specific subject for a specific game activity via website. As shown in Figure 6, therapist chose to display daily maximum hand opening value from game GestureFly on website. The therapist sent a short text messages to the patient whenever there was new training data shown on the server side as a way to praise her effort and encourage continued participation. The data showed that repeated performance of several simulations resulted in increased finger opening as measured by the system.

DISCUSSION

We intend to bring a “wellness” approach to the achievement of independent upper extremity manipulation and function for people post-stroke. The National Wellness Institute (mailto: <http://www.nationalwellness.org>) encourages “a conscious, self-directed and evolving process of achieving full potential.” Through the development of an easy to use and engaging home-based virtual reality exercise program we modeled the wellness concept of continual self-directed exercise to allow people with hand and upper extremity motor deficits to achieve their full potential. NJIT HoVRS is a low cost, easy to setup, reliable and engaging home-based hand rehabilitation system. Virtual reality simulations are engaging and the subject was motivated to play them for a long period of time. Currently, there is little available for home-based hand therapy (10). The advantage of this home-based system is its focus on hand-based simulations. Our objective was to provide markerless hand tracking (LEAP) and simple support of arm motion in 3-dimensional space to allow the patient to move freely, while it helps them keep their hands over the LEAP’s effective range. Clear online directions, delivered visually and aurally through imaginative avatars allow the individuals to practice independently. Novel algorithms provide safe and effective interactions with the serious games. Despite this complexity, the use of novel technologies makes this affordable system easy to use. Compliance with home-based exercise programs is low. We believe strongly that the NJIT-HoVRS home-based system will effectively deal with compliance issues through the engaging games, the easy to use system (no donning and doffing difficulties), the enticing encouraging avatars, the success algorithms and the instant availability of daily achievement graphs. This self-paced, independent home practice is consistent with a “wellness” approach that should empower stroke survivors to take an active self-directed approach to their rehabilitation.

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Chapter 102

FACE TO FACE: AN INTERACTIVE FACIAL EXERCISE SYSTEM FOR STROKE PATIENTS WITH FACIAL WEAKNESS

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ABSTRACT

Each year 152,000 people in the United Kingdom have a stroke. Almost all have an initial facial weakness. Many resolve in the first few days but it is estimated that 26,000 people experience some kind of long-term paralysis in their face. A survey of 107 United Kingdom based clinicians found that routine treatment of facial weakness was provision of exercises with a written instruction sheet. A multidisciplinary team, which includes patients, researchers and therapists have produced a working prototype system to improve facial weakness. It is called Face to Face and includes a Kinect sensor, a small form PC and a monitor. Patients follow exercises given by a therapist on the screen; the system records and simultaneously gives feedback, with a facial recognition algorithm providing tracking data for each captured frame of the user's face. Results from our small clinical trial indicate that the system is more successful at getting patients to complete their

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exercises than using a mirror, patients liked it, and they said it had helped improve their facial symmetry. Therapists said Face to Face encouraged patients to exercise daily, they liked the fact that it could be individually programmed and could record how much the patient had exercised. Based on the initial project work and positive outcomes Face to Face aims to help patients practice their facial muscle exercises to speed their recovery, providing direct benefits in terms of costs and time, and offering patients significant improvements.

INTRODUCTION

The overall aim of the project was to test an effective, engaging and affordable interactive facial exercise system for use with people with facial weakness following stroke. This is to meet patient and clinical needs and lead to measurable improvements in clinical outcomes (1-3).

Face to Face is a computer based system that enhances and automates the therapy provided by Speech and Language Therapists (SLTs). The system is initially aimed at stroke survivors suffering from facial paralysis, particularly with stroke patients suffering from swallowing and speech problems. The project development team also propose that the system may become a 'platform' system and be adapted and used more widely for a number of conditions and associated facial weakness.

A partnership between Nottingham Trent University, Nottingham CityCare Partnership, University of Nottingham, an industrial partner and service users within the UK have produced a system for use in the home, which uses the Microsoft Kinect games interface and a PC monitor to develop an interactive system to assist in the rehabilitation of patients with facial weakness. The aim is that patients will use this system to complete exercise programmes by copying on-screen exercises, and as they do this the system will monitor and *measure* the patient's face. Members of the University of Nottingham Stroke Research Partnership Group confirm the need for facial paralysis rehabilitation; currently there is a lack of appropriate and affordable alternatives in the market.

The system recognises facial expressions by tracking movement across the face and applying the recognised motion onto an onscreen representation of the user. This tool recognises differences between an undesirable one sided (unilateral) movement and a desired symmetrical movement across both sides of the face. This information is then conveyed via the monitor using the form of a graphical representation of the patients face. Using the representation as both a visual and auditory communicator, the system takes the patient through a series of exercises and indicates the degree of success with the exercise programme. An international patent application for the system was filed in 2015 (4).

Patient Benefit

A key aspect of the system is the ability to visualise the improvements that the patient is making from repeated exercises. The system is simple to set up, with no need to attach any systems or markers to the face. The user interface permits small bursts of exercises to be performed throughout the day, rather than undertaking one long and tiring session. The

overall aim of the initial project study was to test an effective, engaging and affordable interactive facial exercise system for use with people with facial weakness following stroke. This is to meet patient and clinical needs and lead to measurable improvements in clinical outcomes. The system has the potential to be of benefit to patients by: improving fidelity in rehabilitative exercises prescribed for facial weakness; providing immediate feedback to patients on their progress; improving patient's facial weakness, including carers in the rehabilitation process (a Stroke Association recommendation); assisting patients in meeting the recommended target of 45 minutes daily therapy and reducing the need for patients to visit hospital for rehabilitation.

Clinical Need

Approximately 16% of people suffering stroke have long lasting facial weakness (5). A combination of physiotherapy, occupational therapy and speech therapy has been proven to be clinically effective in the treatment of those who have suffered facial weakness following a Stroke. However, whereas the National Clinical Guideline for Stroke (6) indicates that patients should receive 45 minutes of therapeutic treatment daily, the majority of patients receive far less than this. The Royal College of Physicians National Sentinel Stroke Clinical Audit (7) states that only 33% of patients receive 45 minutes or more physiotherapy daily and only 18% of patients receive 45 minutes or more speech and language therapy daily.

It is clear from previous studies, and from involvement with partners at Nottingham University Hospital and the Stroke Association, that there are currently insufficient resources within the NHS to provide the necessary level of therapeutic care post Stroke. The most common rehabilitative exercises prescribed to facial weakness sufferers are either practising motions in front of a mirror or using an instructional DVD. These methods cannot be considered as engaging, due to their lack of feedback and quantifiable progression. Less commonly available is an electrical muscle stimulator designed for facial uses. This machine is made by the NHS electronics departments, and is in short supply, with recipients tending to get a maximum of six weeks to use the system before it is given to the next patient. The machine electrically stimulates the affected muscles to the point that they get exercise through contraction. It is reported to work well in the short term, but owing to short supply and the high price of working machines, along with a lot of training required on the part of the user, it is not currently considered a sustainable solution.

Initial System Concepts

Initial system design concepts were based on the utilisation of the patient's own TV with a 'plug and play' 3D system attached. Over a development period of 18 months the preferred options were discussed and agreed with the patient and public involvement group (PPI), resulting in a PPI informed user interface and physical system design based on the Kinect sensor as shown in Figure 1.

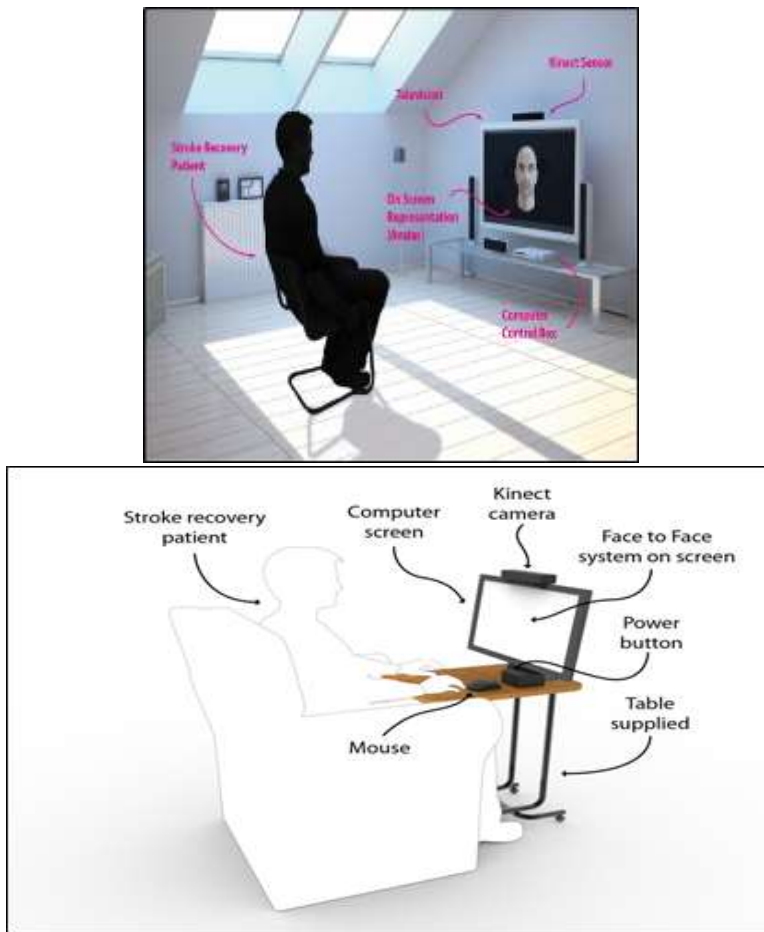


Figure 1. Initial concept (inset) and PPI informed system design.

The system that has been developed as a result of this project recognises facial expressions by tracking movement across the face and applying the recognised motion onto an onscreen representation of the user. How the user performs a series of facial exercises is assessed by the system and scored according to how well the user can do each of a defined set of expressions. The therapist is provided with a record of exercises as they were performed. New movements can be introduced automatically, with incrementally achievable goals throughout the week. The system will enable shorter consultations through the presentation of quantified improvement and empower the carer and the stroke survivors to take on some of the role of supervising the performance of the exercises.

It is proposed that the ‘home rehabilitation’ solution would help offset some of the problems associated with transfer from hospital to home care. The “Moving On” report (8) states that 48% of Stroke survivors believed that community and home-based therapy helped them become less long term reliant on carers.

System Overview

Game based technology platforms are now widely used for physical rehabilitation of patients, recognised as a potential motivational tool for rehabilitation. New opportunities in rehabilitation have clearly arisen with emerging technologies of computer games and novel input sensors including 3D cameras. Hossein and Khademi (9) provided a review on the technical and clinical impact of the Kinect on physical therapy and rehabilitation. Omelina et al. (10) examined serious games for physical rehabilitation and Webster and Ozkan (11) provided a detailed review of elderly care and stroke rehabilitation systems utilising the Kinect. As 3D facial image processing for this research project provides clear additional pose mapping functionality, the benefits of processing depth information when compared to 2D vision systems was explored by González-Ortega et al. (12) in relation to Kinect based systems for cognitive rehabilitation exercise monitoring.

Currently the system has been developed utilising pre-existing facial recognition software known as Faceshift (available from www.faceshift.com and now owned by Apple). Image data is acquired from the Kinect and, when a face is detected, a model is generated of the user's face. A profile can be stored for each user by capturing a pre-set series of expression, with an increased level of accuracy obtained by placing markers on a scanned face. This setup routine may be performed typically by a speech and language therapist (SLT), but should only need to be carried out once, following which the user can operate the system independently.

OUR STUDY

A short review on image processing was conducted by Samsudin and Sundaraj (13) for facial paralysis and facial rehabilitation, identifying image processing as being widely utilised but with very limited applications in relation to facial rehabilitation. Further Xiaozheng and Gao (14) provided a critical review of face recognition techniques and the clear challenges particularly in relation to pose invariant face recognition, whilst Wang et al. (15) examined real time movement linked to an evaluation of skeletal pose tracking using the Microsoft Kinect first and second generation hardware.

Clearly various complications in computer vision require an assessment of pose objects in real-time with numerous reliable solutions available for pose estimation utilising feature-based 3D tracking. However difficulties can arise based on the need for fast and robust detection of known objects in view, for example the Kinect sensors used for this project were notably affected by ambient lighting conditions.

For the Face to Face system, once the facial recognition software is set up to track a user's face the facial recognition algorithm provides tracking data for each captured frame of a user's face. The tracking data relates to a number of facial gestures, together with a value associated with each gesture. In Faceshift, 48 channels of raw tracking data are provided per frame, each of which is associated with a number ranging between 0 and 1. The numbers, or scores, represent the strength of deformation on the 48 predefined gestural channels. For example, the gestural channel 'BrowsD_L' measures lowering of the left eyebrow, and 'LipsStretch_L' measures sideways movement of the left corner of the mouth. These numbers

do not represent physical units, but are relative scores defined by upper and lower limits of each facial gesture. These scores are then used to analyse how well the user is performing a particular facial expression.

The speech and language therapist (SLT) defines a number of facial expressions, the facial expressions being specific to a user's rehabilitation programme. These facial expressions can each be defined as a combination of facial gestures that can be captured by the facial tracking algorithm, with each facial gesture having an associated target score. The overall aim of an exercise programme setup for a user is to encourage the user to perform the facial expressions and have these analysed and scored by the computer, using the facial recognition algorithm. To do this, a measure of how well the user is performing a particular facial expression needs to be defined. The mathematical space of tracking variables obtained from the facial recognition algorithm is mapped into a target space of facial gestures (or 'poses') for a particular facial expression. For each pose a measure termed a 'pose match strength' may be defined, for example as another number ranging between 0 and 1.

Pose match strength can be computed from the source tracking channels by defining a subset of the source channels as 'pose channels' that influence the pose match strength value for the facial expression being assessed. Source channels that are not included in a particular pose definition can either be ignored or treated as 'wildcards' for the match, for which any value will match. These represent channels that are irrelevant to the facial expression being exercised. As an example, mouth poses typically would not depend on eyebrow positions, resulting in the values for any eyebrow-related channels being discounted in an assessment of a mouth-related pose. Included pose channels may each define a target value or score for the pose, typically over the input range of between 0 and 1). This score is used to calculate an individual channel match value (again between 0 and 1). The range of input source channel values, i.e., from a minimum to a target value, is linearly mapped to an output pose channel match of 0 to 1. Beyond the target value the output pose channel match is capped at 1.

Figure 2 shows a pose tracking value over time, as defined by 14 successive samples, with a pose match peak of 0.8 and a peak value defined at 0.7 based on the development of the pose tracking value. During each 'out of pose' phase (while the system is looking for an 'in pose' event), whenever the instantaneous tracking value reaches a new highest value this 'pose match peak' value is recorded, and the time is recorded. A moving average is then maintained over the 'time since last peak', which will start at the peak and gradually decline as the instantaneous match strength diminishes.

Once a predefined time threshold is reached (which may, for example be around 0.5 seconds, and typically less than one second) without a new peak value being observed, an enter pose event is triggered and the current moving average is stored as a match value for the current 'active' pose. Conversely if a new instantaneous peak value is reached before the time threshold is reached, a new peak value is recorded, the timer is reset, and a new moving average measurement begins. This can happen several times before reaching a stable peak, particularly if the signal is noisy and/or the peak value is relatively low. The partially unsuccessful short-duration peaks before the final successful peak are analogous to 'false horizons' that are due to noise in the signal, whereas the moving average represents an adaptive threshold which continually adjusts to varying user ability and tracking response.

Each pose channel included in the pose definition may be marked as either a goal or a constraint. The output pose match strength is a relative score that can be calculated as the maximum of the set of all computed goal pose channel match values, limited by the minimum

of the set of all constraint pose channel match values. This is similar to goal channels acting as logical ‘OR’ and constraint channels acting as logical ‘AND’. The score for each facial gesture may be defined as a ratio of a score for the facial gesture provided by the facial recognition algorithm to the target score for the facial gesture. The score may range between 0 and 1, and be limited to 1 if the ratio exceeds 1.

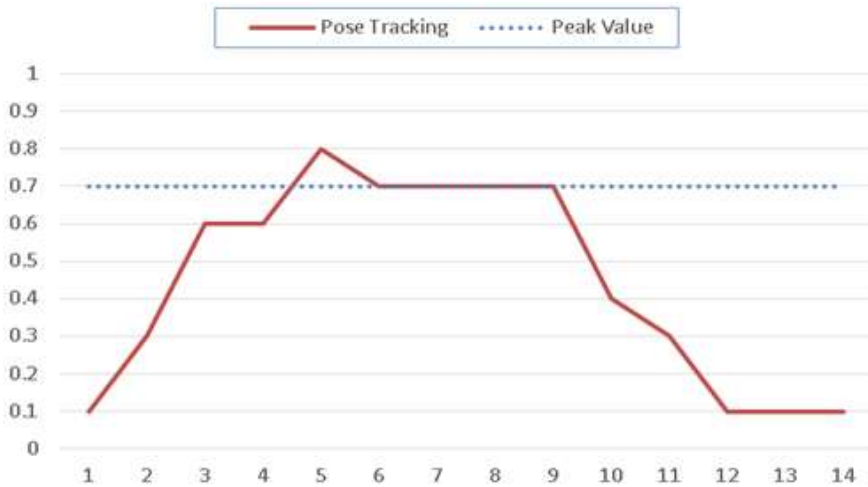


Figure 2. Pose tracking over time (samples 1 to 14).

FINDINGS

Each of the exercise types relies on a predefined series of steps, these steps are illustrated in Figure 3. The process begins with an on-screen instruction phase with an acknowledgement when the number of repetitions has been completed. In between these two phases, the exercise involves looking for a particular target pose at any given time, which may even apply in the case of speed exercises which involve alternating between two poses. When an exercise target pose begins, the state is initially ‘out of pose’ and the system waits for the user to enter the pose. Once in pose, the system waits for the pose match strength to drop below a predetermined threshold value.

The events of entering and exiting a current target pose can trigger different responses depending on the exercise type. For example, speed exercises may switch to an alternate pose on exiting a first pose, whereas range exercises tend to set an ‘anti-pose’ during a relax phase which the user must enter (for example open mouth, followed by closed mouth) before returning to the original target pose for the next repetition (open mouth). Once the user is in pose the user is instructed to hold for a set period, and once the set period elapses the process can then repeat if required or complete. The series of steps involved in performing an exercise transforms a continuous range of pose match strengths into a series of discrete states and state change events. The user interface indicates the number of repetitions, which then triggers the next instructional video (“open your mouth...” / “...and relax”).

A particular problem, however, arises when considering how to determine when a particular pose has been achieved, i.e., when the state should change from 'out of pose' to 'in pose'. This determination can be made because of a continuous assessment of the score for a facial expression that is the subject of the exercise.

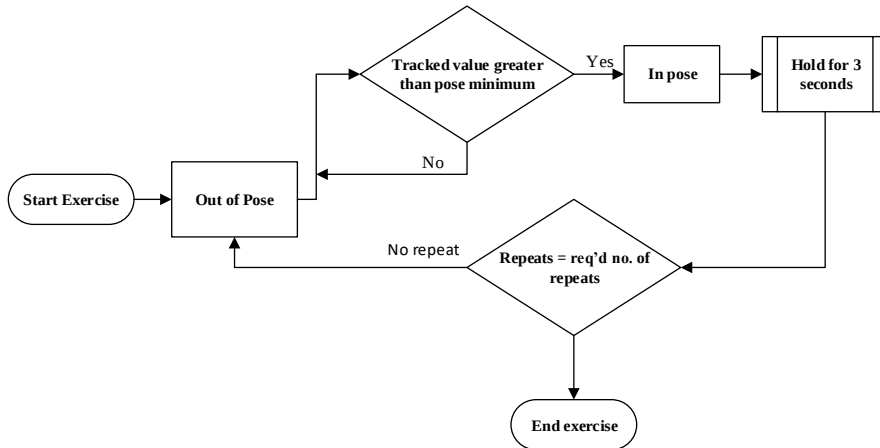


Figure 3. Exercise steps.

Pose Example

The 'ee' pose may be defined by MouthSmile_L (left) and MouthSmile_R (right) as goal channels, each with a target value of 0.8, defining JawOpen as an inverted constraint channel with a target value of 0.5. The following two conditions then result:

Condition 1:

MouthSmile_L = 0.2 - goal match $0.2/0.8 = 0.25$

MouthSmile_R = 0.6 - goal match $0.6/0.8 = 0.75$

JawOpen = 0.3 - inverted constraint match = 1

(less than target 0.5, inverted, capped to 1)

Pose match strength = min (max (goal),

min (constraint)) = min (0.75, 1) = 0.75

Condition 2:

MouthSmile_L = 0.2 goal match $0.2/0.8 = 0.25$

MouthSmile_R = 0.6 - goal match $0.6/0.8 = 0.75$

JawOpen = 0.8 inverted constraint match = $(1 - 0.8) /$

$(1 - 0.5) = 0.2/0.5 = 0.40$

Pose match strength = min (max (goal),

min (constraint)) = min (0.75, 0.40) = 0.40

This example could be interpreted as: an 'ee' pose requiring MouthSmile Left OR Right, AND jaw mostly closed. In the second scenario, the pose match strength is reduced to 0.4 because the jaw constraint is infringed, limiting the overall output value. In more general terms, a relative score for each identified facial gesture in a facial expression is calculated as a

ratio of the score for each identified facial gesture to the corresponding target score for the facial gesture. The score for the facial expression can be calculated based on a maximum relative score for the facial gestures defining the facial expression. Other combinations of goals and constraints can also be determined, depending on the particular facial expression to be exercised and whether any particular constraints need to be considered. For example, a facial exercise regime may be made up from a set of nine different poses. These may be combined using 9 range exercises with 9 strength exercises and with 3 poses used in pairs in ‘fast’ and ‘slow’ versions to make up 6 speed exercises (AB, AC, BC x fast/slow), making a total of 24 exercises to be performed in the exercise regime. A therapist can then make a patient assessment, and simply enable or disable each exercise from this palette of 24 exercises to quickly construct a tailored exercise regime for that user. A screenshot pose is shown in Figure 4 and the patient progress screen in Figure 5.

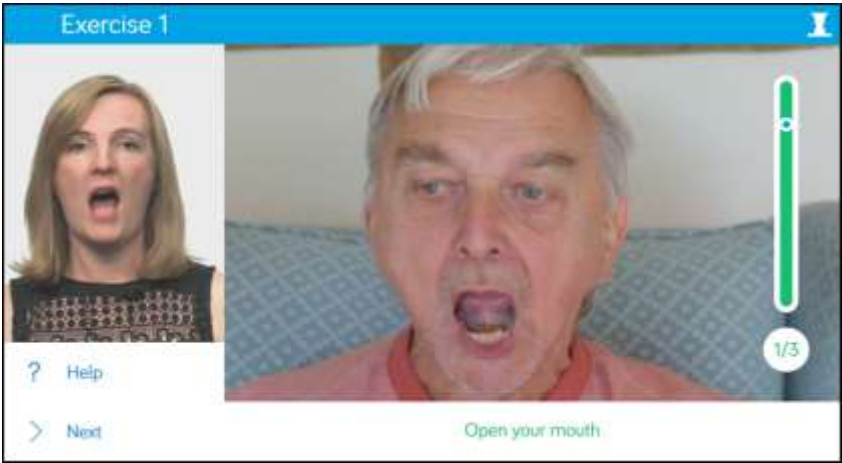


Figure 4. Exercise pose screenshot with a graphical indication of the user's score (right) and exercise number (bottom right) overlaid on a picture of the user next to an image showing the pose to be performed. The pose indicated by a speech and language therapist (left).

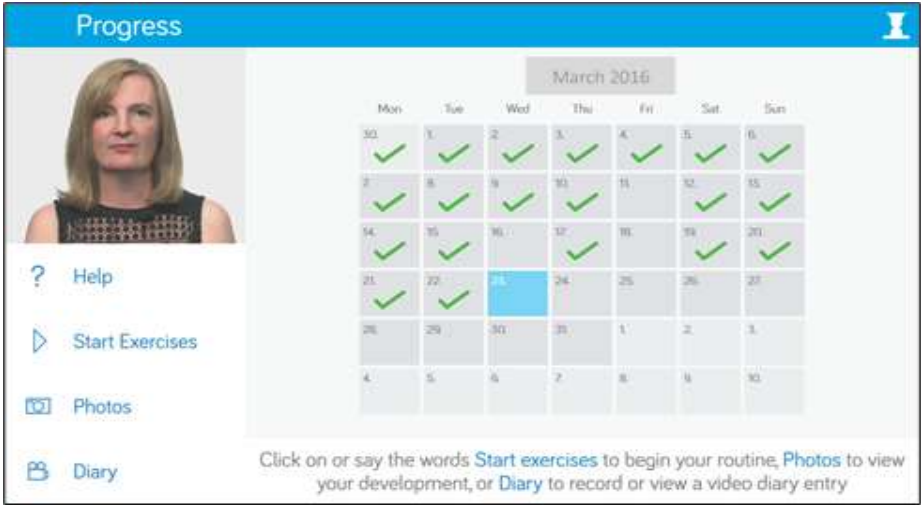


Figure 5. System diary and progress screen.

DISCUSSION

Participants found the Face to Face system to be a user-friendly rehabilitation tool for facial weakness after stroke, usage indicated that all participants were motivated to use the system (16). All participants made a slight improvement from baseline assessment and the therapist observed that: there was possible synkinesis in one participant and there was no deterioration or worsening of this following use of Face to Face system; one participant's ability to speak improved which increased confidence; one participant's natural smile became almost symmetrical and reduced drooling and reduced numbness was reported by one participant, leading to positive comments.

The speech and language therapist (SLT) and all participants noted functional changes and detected clinical changes in the level of facial weakness subjectively. The SLT assessed the level of facial weakness using the Sunnybrook facial grading system (17, 18) and prescribed a treatment plan of facial exercises from the Face to Face programme.

Currently development is focused on transforming tracking data received from the facial recognition algorithm into pose match strengths against a combination of facial gestures, the system enables a straightforward means of constructing and performing an exercise regime. In addition, development of a complex and informative web-based interface for clinical evaluation of patient's use and performance against rehabilitation regimes has been created. This will provide real-time adherence data during rehabilitation and allow a personalised exercise plan to be adjusted remotely, at any time, depending on patient's actual exercise patterns and improvements.

Looking forward, there is a growing number of technologies coming to market that will benefit and enhance this product. Of particular interest is the *new* 3D camera technology launched by Intel. These 3D cameras use the same principles as the Kinect camera but are designed to be integrated into phones, tablets and laptops. Another important feature of the Intel cameras is that they have been specially designed to focus on facial recognition and tracking, this will provide an accurate and reliable facial tracking system for us to use. In contrast the Kinect system is designed for full body tracking, although the system can be used to track facial expressions it is not its primary function and therefore limits the effectiveness of the product and requires a payment for third party software licences.

In current practice, SLTs priorities are in assessing and planning the patient's therapy, and non-professional assistant practitioners (APs) are used on a day-to-day basis with the patient to monitor adherence to the program and assist the patient. We propose to extend the stored usage database to include a record of planned usage regimes for each patient, and to provide a visual comparison of actual usage against the planned regime in the web interface. This approach would allow an AP to interpret the live usage data in the web interface, identifying overuse or underuse of exercises without needing to involve an SLT in the first instance. The web-based interface would still be accessible to SLTs who could perform any further analysis they felt necessary, but they would now also be able to remotely adjust the exercise regime while the device was still in the patient's home. Further development of the product is required and to address other market opportunities, including: improving the user interface to address shortcomings identified in trials and research; improving the compactness and portability of the physical embodiment; strengthening the robustness and functionality of the system (for example in low light conditions); reducing unit cost and finally allowing for a

wider range of exercises, implying a more complex interface requirement to allow SLTs to set up systems for patients.

It is anticipated that this additional functionality would reduce the ongoing costs of deploying the final system within everyday healthcare practice, by reducing the required time from the SLT.

ACKNOWLEDGMENTS

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Chapter 103

STROKE DUE TO VASCULITIS IN ADULTS

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ABSTRACT

The vasculitides are diseases characterized by inflammation of blood vessels and inflammatory leukocytes in vessel walls. There is an increased propensity for ischemic stroke due to the compromise in vessel lumina and distal tissue ischemia, with hemorrhagic stroke and aneurysmal bleeding due to loss of vessel integrity.

INTRODUCTION

The vasculitides are diseases characterized by inflammation of blood vessels and inflammatory leukocytes in their walls. There is an increased propensity for ischemic stroke due to the loss of vascular integrity with stenosis of vessel lumina and distal tissue ischemia; and hemorrhagic stroke and aneurysmal bleeding due to loss of vessel integrity. The 2012 Revised Chapel Hill Consensus Conference (CHCC) [1] nomenclature provides systemic nosology and a categorization of primary and secondary vasculitides. Central nervous system (CNS) involvement and stroke both occur in the single-organ vasculitic (SOV) syndrome primary CNS vasculitis (PCNSV) [2], as well as, due to limited or multiorgan manifestations of primary systemic vasculitides [1].

PRIMARY CNS VASCULITIS

With several proposed diagnostic schemes for PCNSV over the years [3-5], the combination of clinical and histopathological findings derived from cerebral angiography and leptomeningeal and brain biopsy remains the recommended method of definite diagnosis [6]. This approach facilitates the identification of clinicopathologic subtypes with persistent focal deficits and stroke [2] or intracranial hemorrhage [7] from those with reversible cerebral vasoconstriction syndrome (RCVS) [8] that may mimic true vasculitis, but do not require cytotoxic therapy.

Torres and colleagues [9] did not find a significant correlation between brain and leptomeningeal tissue, and the results of cerebrospinal fluid (CSF), neuroimaging, surgical technique, biopsy characteristics, or preoperative immunosuppressive therapy in 9/79 (11%) patients with suspected CNS vasculitis studied between 2005 and 2013. Yet in nearly three-fold (30%) of patients undergoing biopsy, the investigators noted alternative diagnoses of cerebral amyloid angiopathy, encephalitis, demyelination, and lymphoma. Postoperative complications occurred in 16%, 4% of which were serious. The authors concluded that while brain biopsy remains an important diagnostic tool, however further studies are needed to establish the clinical variables associated with a positive yield, as well as the surgical techniques that are most likely to provide diagnostic results with the lowest complication risk.

Historically, Younger and colleagues [3] described four patients with granulomatous angiitis of the brain histopathologically manifesting necrotizing vasculitis of arteries and veins of varying caliber in conjunction with giant cells and epithelioid cells. These authors [3] noted non-focal symptoms of headache and mental change, followed by focal weakness, seizures, and fatal coma in all four patients. Focal weakness with a stroke onset was noted in two (Patient 1 and 3) of the four patients, Patient 1 had onset of lethargy, focal ptosis and hemiparesis had predominant anterior (ACA), middle (MCA), posterior cerebral artery (PCA) and basilar artery involvement at postmortem examination. Patient 3, who presented with hemiparesis, ataxia, and dysarthria, and found to have predominant small leptomeningeal artery and vein involvement. Altogether, 15% of 78 patients overall had focal weakness with stroke onset compared to 42% who presented with focal deficits during the course of the illness.

In the same year, 1988, Calabrese and Mallek [4] described eight patients with classic angiographic or histopathologic features of PCNSV or so called primary angiitis of the CNS (PACNS), and reviewed the literature of 40 literature cases. Patient 2 [9] who presented with the clinical diagnosis of isolated angiitis of the CNS (IACNS) defined by classical angiographic features, had a clinical onset of transient weakness that progressed to fixed hemiparesis, nonfluent aphasia, alexia, and agraphia with later stabilization after combination prednisone and cyclophosphamide therapy.

Birnbaum and colleagues [5] proposed changes to the criteria of Calabrese and Mallek [4] to include a definite diagnosis of PCNSV only after tissue biopsy confirmation, noting strokes or persistent deficits in less than 20% of patients at onset, and with only about 28% of symptoms overall related to large cerebral vessel involvement. Salvarani and co-workers [2] noted that persistent neurological deficit or stroke and headache were the commonest initial symptoms affecting 68% of 101 patients so studied with PCNSV defined by diagnostic criteria similar to those suggested earlier [4]. Cerebral infarction noted on magnetic resonance

imaging (MRI) of the brain in 53% of patients, were multiple in appearances in 85%, bilateral in 83%, and involved the cortex and subcortex in 63%. Overall, their appearance suggested large-artery, branch-artery or small-artery distributions. Intracranial hemorrhage was noted in 8% of patients, and gadolinium enhancement was found in a third of patients, a quarter of whom demonstrated gadolinium enhancement of the meninges.

Montefort and co-workers [10] described a 57-year-old man with brain-biopsy confirmed PCNSV and a 2-month history of right arm weakness and numbness superimposed on a history of left-sided headache and intermittent visual field loss. Brain MRI showed evidence of cortical infarction across more than one vascular territory with signal changes consistent with hemorrhage in the left basal ganglia and parieto-occipital lobe. There was angiographic evidence of irregular narrowing and aneurysmal dilatation of the left terminal carotid artery that stabilized without new areas of new infarction after treatment with intravenous pulsed cyclophosphamide and corticosteroids followed by azathioprine. The neuroimaging features of this patient are shown in Figures 1 to 4, as well as, in a similarly affected patient in Figures 5 and 6.

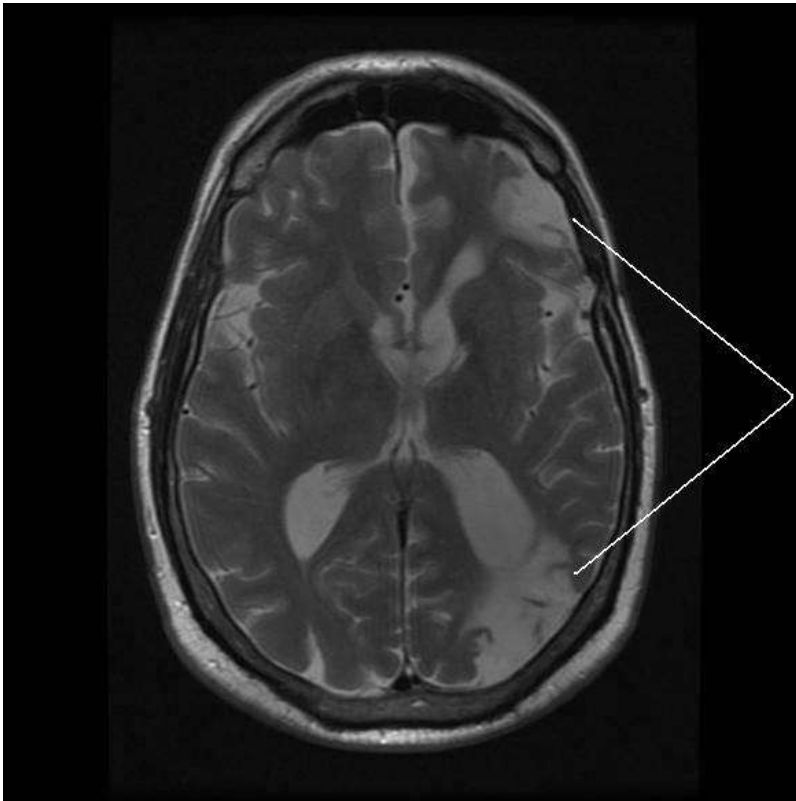


Figure 1. Primary CNS vasculitis. T₂-weighted MRI of the brain demonstrates left frontal and parieto-occipital lobe areas of infarction. Reproduced with permission from [10].

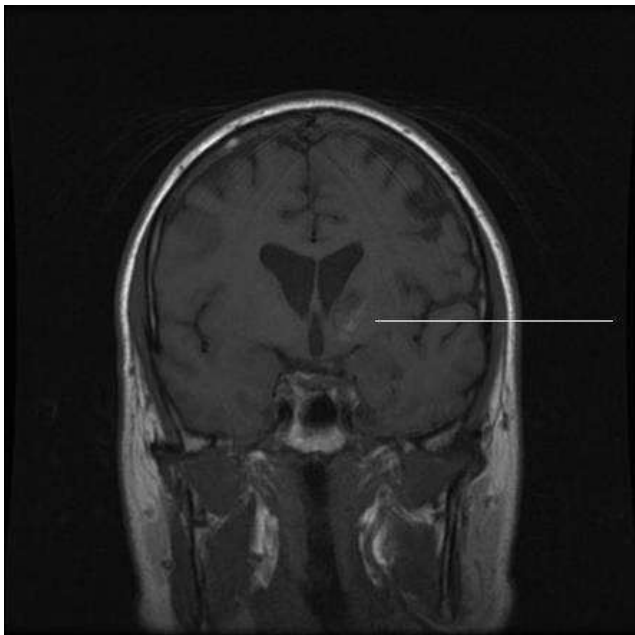


Figure 2. Primary CNS vasculitis. T₁-weighted MRI of the brain showed left basal ganglia hemorrhage. Reproduced with permission from [10].

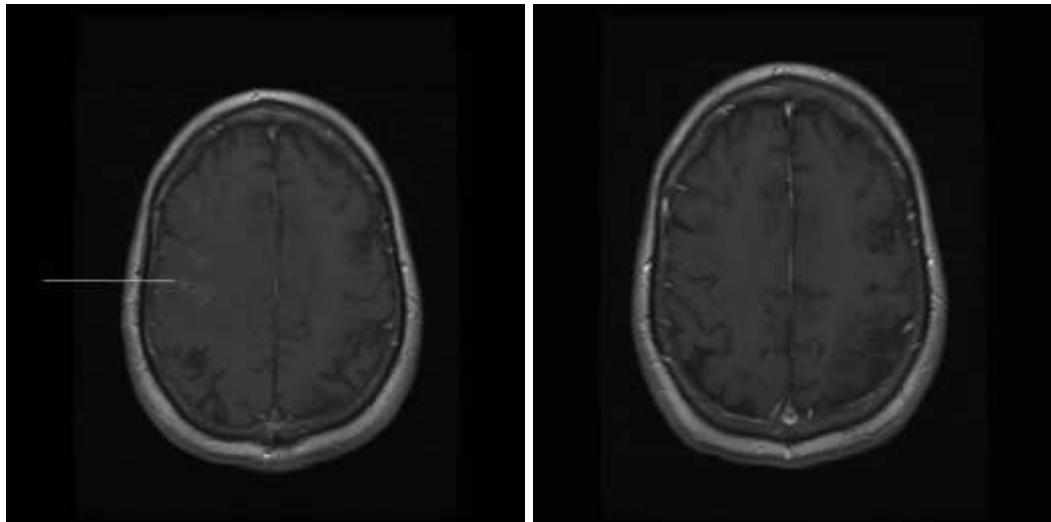


Figure 3. Primary CNS vasculitis. T₁-weighted MRI of the brain demonstrates gyral enhancement after intravenous administration of gadolinium before treatment (A), that resolves after immunosuppressant therapy (B). Reproduced with permission from [10].

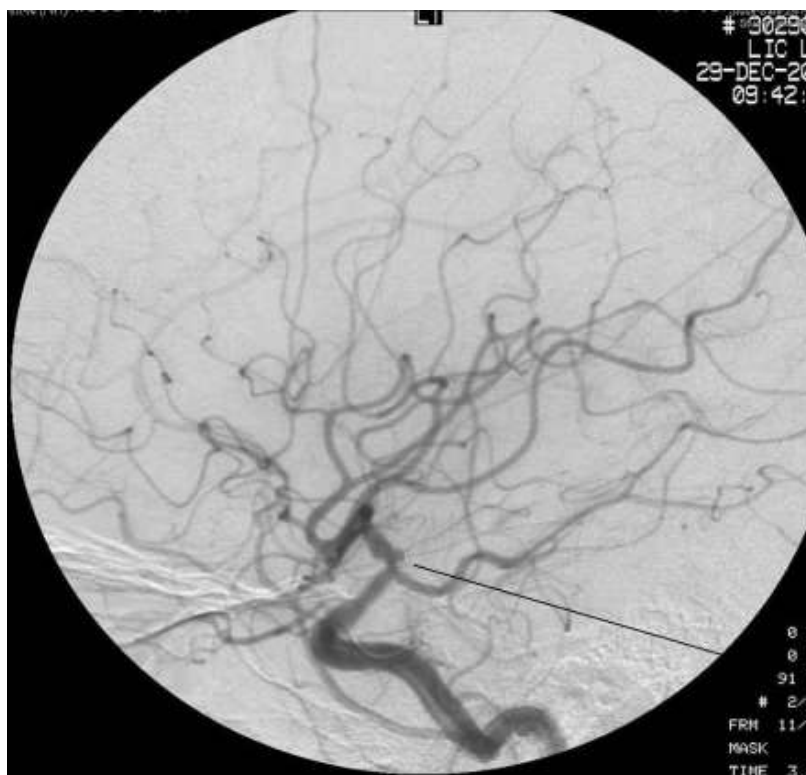


Figure 4. Primary CNS vasculitis. Cerebral angiography shows a sessile internal carotid artery aneurysm. Reproduced with permission from reference 10.

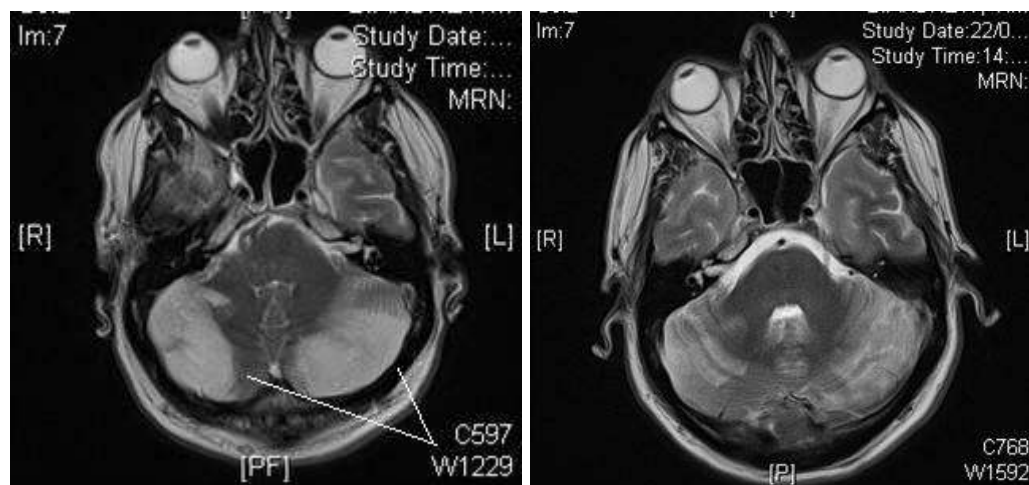


Figure 5. Primary CNS vasculitis. T₂-weighted MRI of the brain shows bilateral cerebellar hemispheric infarcts and abnormal signal hyperintensities before treatment (A) that resolve after immunosuppressant.

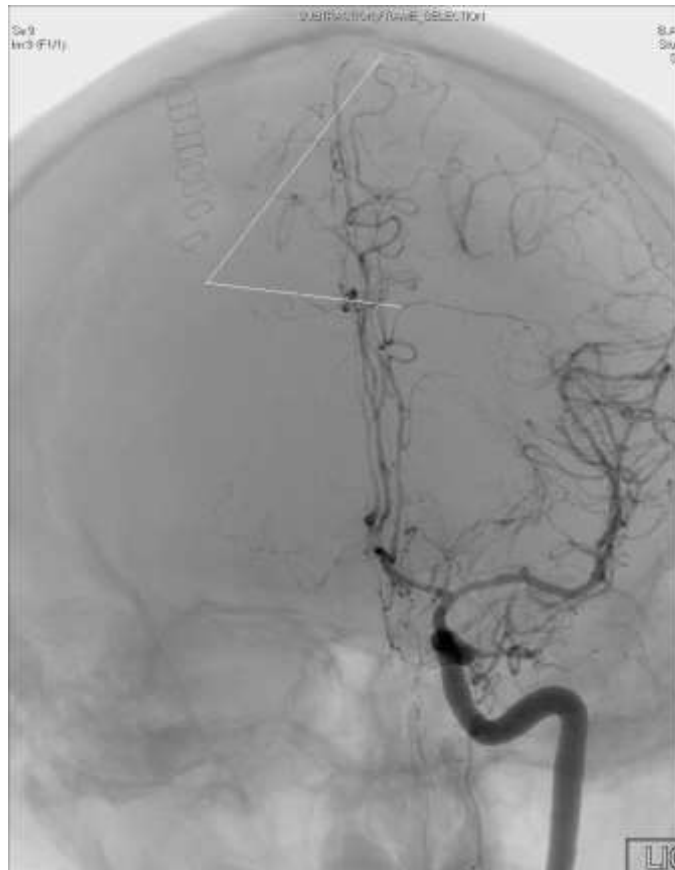


Figure 6. Primary CNS vasculitis. Cerebral angiography shows widespread vessel irregularity and beading.

PRIMARY LARGE VESSEL VASCULITIS

Giant cell arteritis (GCA) and Takayasu arteritis (TAK) are prototypical large vessel granulomatous vasculitides (LVV). They involve the aorta and its major branches however any size artery can be affected [1]. Imaging studies and fluorescein angiography studies have shown that ocular involvement in GCA can affect not only ophthalmic, but also medium caliber retinal arteries and multiple ciliary arteries and their smaller branches; blindness may result from not only LVV but also injury to smaller ophthalmic branches [1]. Whereas GCA has a predilection for branches of the carotid and vertebral arteries and an age of onset older than 50 years, there is often polymyalgia rheumatic. TAK involves proximal subclavian and carotid arteries with an onset of 40 years or less. Neuroimaging studies employing ultrasonography, high-resolution MRI, and ^{18}F fluorodeoxyglucose-positron emission tomography (FDG-PET) imaging are useful modalities to depict superficial cranial and extracranial vessels, and large vessel subclavian and aortic involvement in GCA and TAK [11].

CNS involvement in GCA, which results from thrombosis of the carotid and vertebral arteries rather intracranial arteritis, affects vessels that contain elastin, more specifically the

internal elastic lamina that is absent from intracranial vessels more than 5 millimeters beyond the point of dural perforation. Reich, colleagues [12], Gonzalez-Gay, and co-workers [13] summarized the literature pertaining to stroke and GCA. Reports of CNS involvement have erroneously attributed to intracranial GCA rather than PCNSV [14-16]. Hellenhorset and colleagues [17] noted CNS events including stroke in 7.4% of 175 patients with confirmed GCA, as well as, and one patient with massive cerebral hemorrhage, two with stroke, and six with occlusive disease of the aortic arch. Caselli and colleagues [18] noted transient ischemic attacks or stroke in 7% of 166 patients with biopsy-proven GCA, among whom four had events in the vertebrobasilar system, and eight affected the carotid arterial system. With approximately 30% of patients manifesting neurological findings, the commonest of which were neuropathies of the arms and legs according to Caselli and colleagues [18], Salvarani and colleagues [19] observed that transient ischemic attacks or stroke in the territory of the carotid or vertebrobasilar arteries were less common neurological findings.

Gonzalez-Gay and co-workers [13] ascertained the frequency of cerebrovascular accidents (CVA) in association with GCA among 239 patients in a multicenter retrospective analysis to assess the features, therapeutic response, and predictors of visual loss and CVA. Eight patients (3%) developed CVA, equally divided between the vertebrobasilar and carotid territories. Symptoms of vascular ischemia preceded onset of arteritis by a median of 1.5 months, and were more frequent in those with visual involvement, especially permanent visual loss. Two patients with vertebrobasilar stroke died within one month despite aggressive corticosteroid therapy. Stepwise logistical regression analysis revealed visual loss and jaw claudication as the predictors of CVA.

Kerr and colleagues [20] summarized the clinical, laboratory and treatment responses of 60 patients with TAK based upon the presence of symptoms and signs of ischemic, inflammatory large-vessel disease as well as supportive arteriographic findings, noting ten (17%) patients with either transient ischemic attacks or CVA, and carotid or vertebral artery disease, eight of whom corresponded to the neurological deficit. One of the remaining two had hypertension, whereas the other patient had isolated stenosis of the innominate artery. Riehl [21], and Riehl and Brown [22] reported the clinical and pathological features of six patients with TAK noting widespread arteritis that not only involved the aortic arch and its tributaries by clinical and angiographic studies, but many other medium-, and large-sizes vessels. One patient with TAK manifested clinical and histopathological evidence of CNS involvement. That patient, a 43-year-old woman who presented with rapidly disappearing blood pressure and complained of spells of progressively worsening dizziness and dimness of vision, died of severe congestive heart failure. At postmortem examination, there was severe stenosis of the distal aorta, thrombosis of an aortic arch graft with nearly complete obstruction of all the great vessels by granulomatous panarteritis, as well as vasculitic involvement of the proximal portion of the left MCA, both PCA arteries, and anterior third of the basilar artery. There was massive infarction of the left temporoparietal region, the left half of the midbrain, brain stem, and cerebellum.

PRIMARY MEDIUM VESSEL VASCULITIS

Polyarteritis nodosa (PAN) and Kawasaki disease (KD) are prototypical medium vessel vasculitides (MVV). Although any size of artery is affected in this category, PAN is

associated with necrotizing arteritis of medium or small arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules; however, it is not associated with anti-neutrophilic cytoplasmic antibodies (ANCA) [1]. By contrast, KD is an infantile or childhood disorder that is associated with the mucocutaneous lymph node syndrome, predominantly affecting medium and small arteries, occasionally coronary, the aorta, and other large arteries [1].

Among early postmortem series of PAN, Middleton and McCarter [23] noted CNS involvement due to arteritic lesions in one (Patient 1) that consisted of an occasional small artery. Brain tissue was not examined in one patient (Patient 2) and was not mentioned in another (Patient 3). Kernohan and Woltman [24] summarized the clinicopathological aspects of adult PAN in six patients studied at postmortem examination, estimating CNS involvement associated with stroke in about 8% of cases, and a combination of acute and chronic lesions that correlated with known exacerbations. In three patients, the peripheral nervous system (PNS) was widely involved at onset and at postmortem examination in addition to systemic involvement typically sparing the CNS, while in one patient, the PNS and CNS was involved alone. That patient was a 32-year-old man with onset of left arm and leg hemisensory loss and headache that progressed to paralysis. At postmortem examination, there was marked thickening and near complete obliteration of the right MCA, less so on the left side, and obstruction of the right PCA by a recent thrombus, with a normal PCA on the left. Microscopic analysis showed subacute and chronic lesions of PAN.

According to Guillevin and co-workers [25], CNS involvement is rare but motor deficiencies, stroke and brain hemorrhages can occur as well as cognitive disturbances associated with abrupt memory loss and scattered T₂-weighted hyperintensities on brain MRI suggestive of cerebral vasculitis. CNS involvement including stroke was noted in 9.6% of 115 patients in the FVSG [26] with hepatitis B virus (HBV)-PAN between 1972 and 2002. The FVSG treatment protocol for HBV-PAN generally included cyclophosphamide, corticosteroids and plasma exchange (PE), alone or together, followed by vidarabine, interferon-alpha (INF- α), or lamivudine, depending upon the time of diagnosis and different treatment protocols available [27-29]. So treated, nine patients (9.7%) who achieved remission subsequently relapsed, one of whom died of stroke, although the relationship between PAN and stroke was not clearly established. A retrospective study of 348 adult patients registered in the FVSG [30] who satisfied criteria for the diagnosis of PAN between 1963 and 2005 noted CNS involvement including stroke in 4.6% of patients overall, with a relatively equivalent frequency among patients with and without HBV-related illness.

PRIMARY SMALL VESSEL VASCULITIS

Small vessel vasculitis affects intraparenchymal arteries, arterioles, capillaries, and venules, however medium-sized vessels and veins are also be involved [1]. Two major categories of SVV include ANCA-associated vasculitis (AAV) characterized by necrotizing vasculitis with few or no immune complex (IC) deposits predominantly affecting small arteries, arterioles, capillaries, and venules associated with myeloperoxidase (MPO) ANCA or proteinase 3 (PR3). Marked vessel wall deposits of immunoglobulin (Ig) and complement characterize immune complex vasculitides. Granulomatosis with polyangiitis (GPA)

(Wegener granulomatosis [WG] type), eosinophilic granulomatosis with polyangiitis (EGPA) [Churg-Strauss syndrome [CSS]], and microscopic polyangiitis (MPA) (microscopic polyarteritis) are prototypical AAV, while cryoglobulinemic vasculitis (CV), IgA vasculitis/Henoch-Schönlein purpura (IgAV/HSP), and hypocomplementemia urticarial vasculitis (HUV) associated with C1q antibodies comprise the IC vasculitides; HSP/IgAV is predominantly a disorder of childhood.

ANCA-ASSOCIATED VASCULITIS

Granulomatosis with Polyangiitis

GPA leads to necrotizing granulomatous inflammation of the upper and lower respiratory tracts associated with necrotizing vasculitis of small to medium arteries, arterioles, capillaries, veins, and venules together with glomerulonephritis [1]. Drachman [31] described a patient with one month of headache that awakened him from sleep followed by rhinitis, nasal obstruction, epistaxis, mononeuropathy multiplex, confusion and hypertension. Active arteritis and necrotizing granulomata were found in the brain but not in peripheral nerves. Based upon the observations in a single postmortem studied patient, cerebral involvement according to Drachman [31], depended upon a combination of four separate entities, 1) Frank vasculitis of larger arterial branches particularly over the surface of the brain as demonstrated in his patient report; 2) Ischemia in portions of the territory supplied by the affected vessels; 3) Subarachnoid hemorrhage and hypertensive encephalopathy evidenced by microscopic infarcts in close relation to arteries with fibrinoid impregnation of their walls; and 4) Meningeal infiltration by mononuclear cells. Nishino and colleagues [32] described neurological involvement in 34% of 324 consecutive patients with GPA at the Mayo Clinic between 1973 and 1991 classified according to the American College of Rheumatology (ACR) [33] that included peripheral neuropathy in 16% compared to cerebrovascular events in 4% at some time in the course of illness, however rarely if ever at presentation. Of five patients with presumed clinical vasculitis, cerebral angiography was negative in two. Of twelve patients studied at postmortem examination, findings of vasculitis were found in only two. Fauci and colleagues [34] noted nervous system involvement in 22% of 85 patients, 10 patients (12%) of whom had CNS involvement including strokes at some point in the illness although not specifically mentioned at presentation. Moore and co-workers [35] estimated the frequency of neurological abnormalities of GPA at 23% to 50%, citing possible mechanisms of neural dysfunction due to inflammation from primary sites, remote granuloma formation, and vasculitis. Drachman [31] opinioned that contiguous extension of necrotizing granulomas might account for 24% of all neurological complications including those of the CNS.

Extensive cerebral infarction in the territory of the bilateral ACA was ascertained in a 67-year-old man with frontal headache that preceded detection of a large midline frontal mass lesion on computed tomography (CT), and known biopsy-proven GPA and granulomatous vasculitis of the nasal cavity, liver and kidney. Postmortem examination showed cerebral infarction in the areas supplied by A2 branches of both ACA, with occlusion of large sized ACA vessels by organized mural thrombi, and fibrinoid necrosis in the arterial branches. There was similar vasculitis with multinucleated giant cells involving small arteries and veins

diffusely in the frontal region of the brain suggesting contiguous spread from granulomatous nasal cavity lesions. Hoffman and colleagues [36] retrospectively assessed 180 patients with GPA for 6 months to 24 years noting nervous system involvement in 23% of patients, including CNS involvement and stroke in 8% of patients. A prospective analysis of clinical, electrophysiologic, radiologic, and serologic data of 128 GPA patients over 19 months [37] noted CNS involvement in 7%, the latter including granulomatous infiltration of frontobasal cortex arising from adjacent paranasal sinus granulomas, cerebral vasculitis, stroke, vascular myelopathy, and meningeal granulomatosis.

Microscopic Polyangiitis

Microscopic polyangiitis is a necrotizing AAV vasculitis with few or no IC deposits and commonly associated glomerulonephritis without granulomatous inflammation [1]. Davson and colleagues [38] separated fourteen postmortem studied patients from 1934 to 1947 with MPA into two groups based upon the presence of severe widespread glomerular damage. Wainwright and Davson [39] described MPA among six post-mortem studied patients from 1947 to 1948, mentioned CNS involvement. However, neither paper [38, 39] recorded stroke or histopathologic evidence of brain involvement. Nor was there mention of patients with CNS involvement by Adu and co-workers [40] among 43 patients with renal histologic evidence of MPA. Savage and co-workers [41], who studied 34 patients with MPA, all of whom presented with clinical evidence of a systemic SVV predominantly affecting the skin and musculoskeletal system associated with focal necrotizing glomerulonephritis, cited CNS involvement at presentation in 18% of patients without specific mention of stroke. Serra and colleagues [42], who reported the presentation, histopathology and long-term outcome of 53 patients with MPO from 1965 to 1981, cited CNS involvement at presentation in 15% of patients, the findings of which included stroke, convulsions, headache, confusion and drowsiness. Guillevin and co-workers [43] who reported the clinical and laboratory findings in 85 patients from the FVSG with MPA from 1969 to 1995, noted CNS involvement in 11.8% of patients at presentation and in 3% of 29 patients who experienced a relapse; however, there was no specific mention of stroke. Gayraud and co-workers [44] analyzed four prospective trials that included 278 patients with PAN, MPA and EGPA from 1980 to 1993, that reported stroke occurrence that accounted for 85 deaths. Villiger and Guillevin [45], who reviewed several retrospective European patient cohorts [40-43, 46], cited CNS involvement that included subarachnoid hemorrhage, cerebrovascular disease, meningitis and diffuse brain injury. Ben Sassi and colleagues [47] described intracerebral hemorrhage secondary to necrotizing vasculitis that similarly involved the nerves and muscles, citing three additional patients with hemorrhagic stroke due to MPA [48-50]. Ahn and colleagues [51] studied 55 patients with MPA, 69% of whom demonstrated perinuclear ANCA by immunofluorescence (IF) or MPO ANCA-seropositivity, noting CNS involvement in 5 (9.1%), none of whom developed end-stage renal disease. and one of whom died at follow-up without known autopsy.

Eosinophilic Granulomatosis with Polyangiitis

EGPA is a necrotizing vasculitis involving small to medium vessels that differs from GPA by the presence of eosinophil-rich necrotizing granulomatous inflammation of the respiratory tract in association with asthma and eosinophilia, and ANCA seropositivity when glomerulonephritis is present [1]. Churg and Strauss [52] described the clinical and postmortem findings of thirteen patients with asthma, fever, and hypereosinophilia. This was accompanied by eosinophilic exudation, fibrinoid change, and granulomatous proliferation that constituted the so called allergic granuloma. The latter were found within vessels walls and extravascular connective tissue of major organ systems. CNS organ manifestations were described in 8 (61.5%) of patients, and the cause of death in 3 patients who sustained cerebral hemorrhage or subarachnoid hemorrhage. Chumbley and co-workers [53] described 30 asthmatic patients from the Mayo Clinic over the period from 1950 to 1974 with necrotizing vasculitis of small arteries and veins, extravascular granulomas and infiltration of vessels and perivascular tissue eosinophilia. Lanham and colleagues [54], who emphasized that the combination of necrotizing vasculitis, tissue infiltration by eosinophils and extravascular granulomas suggested by Churg and Strauss [52] occurred contemporaneously in only a minority of patients, and that such histological findings could be encountered in other granulomatous, vasculitic and eosinophilic disorders in the absence of clinical asthma, allergic rhinitis, sinusitis, pulmonary infiltrates, and cardiac involvement pathognomonic of EGPA, noted CNS involvement in 25% of cases. Cerebral hemorrhage and infarction as a consequence of vasculitis or hypertension was a major cause of morbidity and mortality, accounting for 16% of deaths.

Sehgal and colleagues [55] noted neurological involvement among 14 patients with the clinical diagnosis of EGPA, three of whom had cerebral infarction two to fifteen years after initial diagnosis. The first patient was a 68-year-old woman with a stroke involving the territory of the MCA manifested as hemiparesis and aphasia. The second patient was a 34-year-old man with a right parietal lobe infarction pursuant to embolization of a left ventricular thrombus, manifested incoordination and hemisensory loss of the arm. The third patient was a 62-year-old woman with a thalamic infarction who manifested hemibody sensory loss. Guillevin and co-workers [56] studied 96 patients with EGPA between 1963 and 1995, noting ischemic stroke onset in 6 (6%) by brain computed tomography (CT). Mouthon and colleagues [57], reported 38 patients with EGPA from 1978 to 1998, citing CNS involvement in 3 elderly patients (7.9%) in whom CNS vasculitis was suggested by MRI of the brain in only one patient. Among 383 patients with EGPA enrolled in the FVSG Cohort [58] employing the definition of the ACR [59], CNS involvement was noted in 20 (5.2%) of patients at onset, with two-fold greater frequency of ANCA-seropositive serology than those ANCA-seronegative, but no specific mention of stroke frequency.

IMMUNE COMPLEX VASCULITIS

Cryoglobulinemic Vasculitis

Cryoglobulinemic vasculitis (CV) or CryoVas is typified by cryoglobulinemic IC deposited along small vessels predominantly arterioles, capillaries, veins, and venules in

association with serum cryoglobulins. In mixed cryoglobulinemia (MC), the immune complex is composed of IgM and polyclonal IgG with rheumatoid factor activity often in association with hepatitis C virus (HCV) infection. Disease manifestations of cryoglobulinemia was noted in 206 of 443 (47%) patients [60] that included palpable purpura, 12 (6%) of whom had CNS involvement; HCV Infection was the main etiologic factor noted overall in 75% of the 443 patients. A concomitant autoimmune disorder was noted in 24%, hematologic disease in 7%, and essential cryoglobulinemia in 11% of the patients overall. The original patient described by Lerner [61], a 56-year-old man who presented with chest pain, purpuric rash and a cold precipitating serum protein, did not have symptoms or postmortem findings referable to the nervous system. Marshall [62] noted widespread cerebral purpura with hemorrhage ascribed to occlusion of small blood vessels by eosinophilic protein precipitates that correlated with the clinical presentation of progressively fatal coma.

Abramsky and Slavin [63] described three patients with MC and CNS involvement. The first was a 55-year-old woman with anarthria, hyperreflexia, bilateral Babinski signs and progressively fatal coma, who was later found at postmortem to have multiple thrombotic occlusions of small intracerebral blood vessels with adjacent foci of ischemia and marked demyelination. The second patient was a 50-year-old woman, who presented with a Wallenberg syndrome and was found to have MC with occlusion of the left posterior inferior artery at angiography. The third patient, a 50-year-old woman who presented with right hemiparesis and pyramidal signs, was found to have a cryoprecipitate and occlusion of the left MCA.

Gorevic and colleagues [64] summarized the clinical aspects of long-term followup of 40 patients with MC noting incidental CNS involvement at postmortem examination and a fatal stroke in another patient. Petty and co-workers [65] described a 35-year-old woman with type II cryoglobulinemia, headache, purpura, and seizures who was found to have multiple areas of T₂ and proton density signal abnormalities in the cerebral and cerebellar hemispheres, with a gyral pattern of enhancement in the cerebral cortex, a nodular pattern of enhancement in the cerebellum, and early cortical infarction on right frontal brain biopsy without vasculitis; cerebral angiography was normal. HCV RNA was detected by polymerase chain reaction (PCR). Ince and colleagues [66] described a patient with paranoid psychosis and MC who was found to have right basal ganglia hemorrhage and multifocal small vessel occlusions by amorphous bland protein plugs and abundant Russell bodies present in arterioles and venules with relative sparing of capillaries.

Stroke was the presenting feature of CNS involvement in MC among two patients with lacunar infarcts and associated subcortical white matter changes on brain MRI [67, 68], and in one patient with a temporal arteritis-like syndrome with associated ischemic cerebral infarction [69]. Cacoub and co-workers [70] compared the features of patients with HCV examined for systemic vasculitis manifestations of PAN or MC noting two patients with cerebral vasculitis in the former versus none in the latter. Cacoub and colleagues [68] described CNS involvement in three patients with HCV infection and MC, one of whom was found to have a hemorrhagic lesion of the external capsule in association with pontine T₂ hyperintensities and parietal lobe infarcts. A second patient had a hemorrhagic occipital lobe lesion in association with cerebellar infarction and abnormal periventricular hyperintensities. A third patient had abnormal white matter hyperintensities.

Ferri and colleagues [71] studied the demographic, clinical, serologic features and survival of 231 patients with MC seen between 1972 and 2001, noting widespread vasculitis involving small- to medium-sized arteries, capillaries and venules with two or more visceral organs including the kidney, gut, and lung, noting no CNS manifestations of MC with exception of focal dystonia during interferon treatment in one patient [71]. Filipini and colleagues [72] reported a 63-year-old woman with acute severe encephalopathy HCV-related MC, dysarthria and hemiplegia. Fragoso and co-workers [73] reported a 35-year-old woman with dysarthria, left facial and left limb hemiparesis and hemisensory loss with MC in whom MRI showed ischemic lesions in the tail of the right caudate nucleus, corona radiata and posteromedial putamen. Casato and co-workers [74] reported the CNS findings in 40 patients with HCV-related CV in a multicenter, case-control study using MRI and neuropsychological testing. Although none evidenced cerebral infarction, small white matter lesions were found in all HCV-related MC subjects, a higher mean number of white matter intensities compared to HCV and healthy controls.

Terrier and co-workers [75] analyzed the data of 242 patients registered in the CryoVas survey ascertaining CNS involvement in 5 (2%) patients without specifying whether stroke was encountered. Terrier and Cacoub [76] reviewed CNS findings in HCV-seronegative and seropositive MC in CryoVas noting 2 (0.7%) patients among the former with CNS involvement, compared to 9 (5%) patients in the latter group evidencing CNS involvement but without further mention as to presence of stroke in any of the patients.

Hypocomplementemic Urticarial Vasculitis/C1q

Hypocomplementemic urticarial vasculitis/C1q presents with recurrent attacks of erythematous, urticarial and hemorrhagic skin lesions lasting up to 24 hours at a time, associated with recurrent attacks of fever, joint swelling, and variable abdominal distress. Circulating hypocomplementemia and C1q antibodies are associated with necrotizing SVV in skin biopsy tissue affecting arterioles, capillaries, and venules [1]. Apart from orbital pseudotumor with ptosis, diplopia, and headache in one patient with HUV syndrome, there was no mention of CNS involvement or stroke in a review of 18 patients with HUV/C1q [77]. Nor was stroke mentioned in a review of four patients studied at postmortem examination with HUV/C1q [78]. Buck and colleagues [79] cited rare CNS manifestations in HUV/C1q including aseptic meningitis, pseudotumor cerebri, transverse myelitis but no mention of stroke or cerebral vasculitis. Grotz and colleagues [80] did not mention stroke or CNS vasculitis in a case report and review of the literature of HUV/C1q. In a review of HUV/C1q, Davies and Brewer [81] cited CNS involvement as “very rare”, attributing development of pseudotumor cerebri to vasculitis of the venous sinus system. Ludivico and colleagues [82] suggested that chronic pseudotumor cerebri in their patients was a diagnosis of exclusion and possibly related to chronic corticosteroid use, but did not cite patients with cerebral vasculitis or stroke.

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Chapter 104

DIRECT AND INDIRECT BENEFITS OF TRANSLINGUAL NEUROSTIMULATION TECHNOLOGY FOR NEUROREHABILITATION OF CHRONIC STROKE SYMPTOMS

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ABSTRACT

Objectives: The original feasibility study discussed within this manuscript aimed to evaluate the scope of applications for a novel neurorehabilitation intervention. This retrospective piece was composed several years after the completion of the study and focuses on two interesting subjects in particular whose opposing symptomatologies represent the vast spectrum of chronic symptoms after stroke. The present paper brings to light important observations from that initial study and considers possible applications of TLNS Technology for the future.

Materials and Methods: Participants (N = 5) enrolled into a 13-month clinical intervention comprised of three components; (1) translingual neurostimulation (TLNS) using the PoNSTM device, (2) targeted training, and (3) physical exercise. These components will be elaborated on later in the paper. For the first six months of the intervention the participants practiced all three components for one hour, twice daily. Following a thirty-day withdrawal period, participants resumed exercise, training, and device use for an additional six months. Measurements of observation included the Sensory Organization Test (SOT), Dynamic Gait Index (DGI), Timed Up and Go (TUG) test, Stroke Impact Scale (SIS), Dysarthria Impact Profile (DIP), and the Quick Inventory of Depressive Symptoms, Self-Report (QIDS-DR16).

Results: Two specific cases were selected because of their demonstrability that TLNS Technology, as a platform technology, is much wider than previously thought, thus opening more doors in the direction of integrative neuroscience and combination therapy.

Conclusion: We hypothesize that the beneficial effects observed resulted from lasting and cumulative neuroplastic changes (functional, synaptic, and neuronal) in the

brainstem and cerebellum at the cellular and neural network levels, elicited by powerful flow of neural impulses (spikes) from the tongue. The original feasibility study importantly breaks ground for scientific inquiry regarding the limits of rehabilitation and improvement of symptoms for individuals in the chronic stage of stroke recovery. The observations reported here present an opportunity for applications of a new non-invasive brain stimulation technique in the applied and rehabilitative neurosciences.

Keywords: neurostimulation; stroke; rehabilitation; case series; motor dysfunction; post-stroke

1. INTRODUCTION

In 2010 the Tactile Communication and Neurorehabilitation Laboratory (TCNL), at the University of Wisconsin, Madison, conducted a feasibility study ($n = 5$) with the goal of evaluating the scope of applications for a novel neurorehabilitative technique for treating the chronic symptoms of stroke: the combination of targeted training, physical exercise, and translingual neurostimulation (TLNS). This targeted training involved balance and gait training on a treadmill, which was administered by a licensed practitioner (physical therapist). Physical exercise referred to stretching, power, and movement control exercises, also under the guidance of a physical therapist. The physical exercise component can also be thought of as physical conditioning, whereas the targeted training focuses on the specific deficits of the participants—in this case balance, gait, and mobility rehabilitation. For participants with balance and vestibular disorder these tasks are quite challenging. The stimulation itself, administered by a small instrument called the Portable Neuromodulation Stimulator (PoNSTM) device, delivers electrical stimulation to the brain via cranial nerve endings in the tongue – hence, *translingual* neurostimulation (TLNS). This three-part intervention was developed in the early 2000's originally by the name of Cranial Nerve Non-Invasive Neuromodulation (CN-NINM). Previous studies [20, 21] showed that this intervention evoked changes in brain activation patterns on fMRI in response to optic flow stimulus in regions associated with movement, balance, and various integration networks.

The present paper will focus on the results from two of the five subjects who presented with opposing symptomatology because they represent the broad spectrum of chronic stroke symptoms. One of the participants presented with above-normal cognitive function but deficient balance and gait, while the other presented with normal physical condition yet suffered from severe emotional dysregulation and speech problems. While the three other subjects also demonstrated improvement, this discussion will focus on the two that we believe best demonstrate the breadth of applicability of the intervention. The purpose of showcasing these two individuals is to make two key points: firstly, that the same intervention, which was successful in recovering balance and gait for individuals with vestibular disorder [22, 23] and multiple sclerosis [24, 25] in previous studies, works equally well when applied to symptoms of chronic stroke. Secondly, many other symptoms were improved in addition to balance and gait, subsequently expanding the package of symptoms that can potentially be treated using TLNS Technology to include cognitive and emotional disorder.

The primary goal of the feasibility intervention was to recover balance, gait, and mobility. These specific cases, however, demonstrate that the potential for application of

TLNS Technology is much wider than previously thought, thus opening more doors in the direction of integrative neuroscience and combination therapy.

All participants, who were several years post-acute, completed a standard prescribed stroke rehabilitation program and were deemed maximally recovered by a medical professional prior to enrollment in this study. Improvements in balance, gait, and mobility were measured using the Sensory Organization Test (SOT), Dynamic Gait Index (DGI), and Timed Up and Go (TUG) test. Measures of cognitive and emotional recovery included the Stroke Impact Scale (SIS), Dysarthria Impact Profile (DIP), and the Quick Inventory of Depressive Symptoms, Self-Report (QIDS-DR16). Tables 1 and 2 illustrate the specific measures and percent improvement for participants #1 and #2, respectively.

2. RESEARCH DESIGN AND CONTEXT

For multiple years, the TCNL engaged in discovering possible solutions for sensory and motor disorders. The goal of TCNL's research is to enhance the rehabilitation process by improving upon existing therapies and developing new ones for conditions, which, in the past, had few or no options. This clinical, IRB approved study consisted of a 13-month intervention, comprised of three primary components; (1) translingual neurostimulation using the PoNSTM (Portable Neuromodulation Stimulator) device, (2) targeted training, and (3) physical exercise.

For the first six months of the intervention these individuals practiced all three components for one hour, twice daily, followed by a thirty-day withdrawal period, and an additional six months of resumed exercise, training, and device use. This intervention design is informed by an accumulating body of evidence on the positive and compounding effects of integrative, multi-modal therapy [1-5].

3. RESULTS

3.1. Direct Benefits of Physical Rehabilitation

3.1.1. Recovery of Balance

According to the National Council on Aging, falling is the leading cause of fatal injury and the most common cause of nonfatal trauma-related hospital admission among elderly adults, resulting in 2.8 million injuries treated annually in emergency departments. Recovery of motor coordination and balance can decrease this danger dramatically. We used the Sensory Organization Test (SOT) to quantitatively measure participants' ability to use visual, proprioceptive, and vestibular cues to maintain postural stability [6]. One subject (referred to as Subject 1) improved 20.97% from baseline to the end of the intervention, from *substantially impaired* (greater than one standard deviation below the mean) to *normal*. This qualifies as a significant change and is attributable to the effects of the rehabilitation intervention [7].

A tremendous amount of this improvement occurred in the first two weeks and then was maintained through the end of the intervention, with the exception of a slight performance

drop after washout. This supports a possible stimulation dependence and retention effect, discussed in a previous publication [8].

We attribute these changes to the intervention and TLNS specifically because, as previously stated, all subjects enrolled in the study were in the chronic phase of their recovery after having undergone classically prescribed interventions, and were deemed maximally recovered by medical professionals as part of the study’s inclusion standards., Participants reported experiencing increased abilities to perform activites such as shopping, housework and grooming as well as a renewed sense of independence as a result of balance recovery. Furthermore, all participants expressed increases in their capacity to work and participate in social activities.

Table 1. Direct Benefits of TLNS Intervention on Balance, Gait, and Mobility Rehabilitation

Participant 1	SOT	DGI	TUG	Strength	Hand Function	Mobility	ADL/IDL	Social Participation
Baseline	62	14	15.6	56.3	20	66.7	55	37.5
End of Intervention	75	23	10.7	75	75	88.9	75	90.6
Percent improvement	20.97	64.29	31.41	33.20	275	33.30	36.40	141.60

Table 1 depicts scores and percent improvement between baseline and the end of the intervention for subject 1 on measures of physical and vestibular rehabilitation. SOT, DGI, and TUG are separete measurement tools. Strength, Hand Function, Mobility, ADL/IDL, and Social Participation are subscores of the Stroke Impact Scale (SIS).

3.1.2. Gait Normalization

The Dynamic Gait Index (DGI) DGI was developed as a clinical tool to assess gait, balance, and falls risk. It evaluates not only usual steady-state walking but also gait during challenging tasks. Subject 1 recovered 64% from baseline on this measure, eventually reaching the ceiling of this test. At enrollment, this individual presented with a deficient walking score of 14, which is consistent with scores reported in prior research on chronic stroke [9]. Individuals with vestibular disorders who score 19 or less on the DGI are 2.58 times more likely to have reported falling in the previous six months [10]. However, after only two weeks of treatment this individual’s risk of falling was dramatically reduced, recovering to a score of 18.

This improvement was also maintained for several weeks before dipping when stimulation was removed; again demonstrating stimulation dependence during the brief washout period. Improvement on the DGI satisfied both the Minimal Detectable Change (MDC) [11] and the Minimally Clinically Important Difference (MCID) criterion [12].

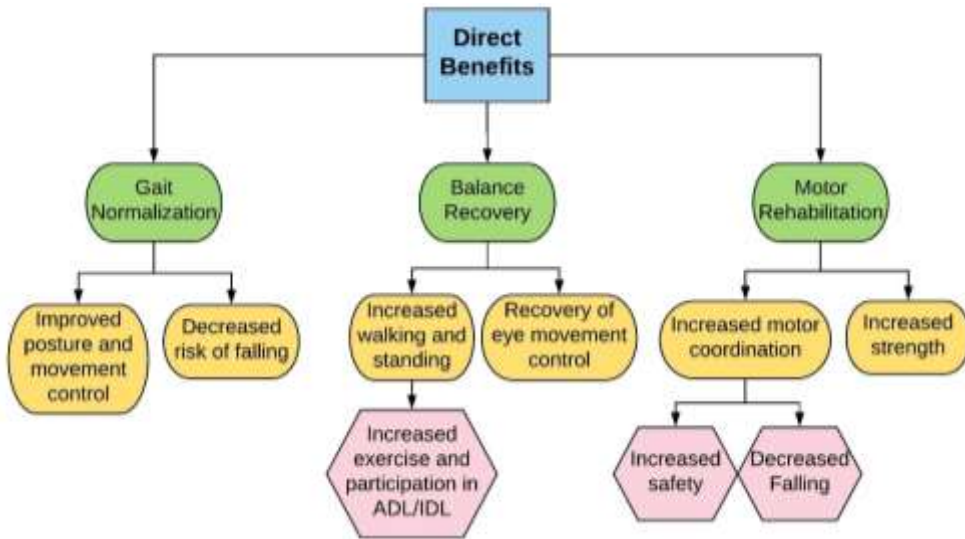


Figure 1: Map of direct benefits of TLNS Technology captured from quantitative assessments, clinical evaluations, and participant feedback

3.1.3. Mobility Rehabilitation

The Times Up and Go (TUG) task is a measure, in seconds, of rising from a chair, walking in a loop, and sitting back down. This task enables the assessment of a participant's coordination and weight transfer, and is challenging for people with balance and vestibular deficits for obvious reasons. TUG time for Subject 1 decreased by 31%, evidencing that their ambulation, coordination, and balance significantly improved as a result of the intervention [13].

3.2. Indirect Benefits of Mental Rehabilitation

3.2.1. Recovery of Emotional Control

Emotional control was the primary concern of the other participant at the time of enrollment (referred to as Subject 2). Her self-reports included feelings of being out of control of her emotions; with frequent high and low mood swings. She was particularly perturbed by her unshakable depression, which had persisted since the time of her injury. To observe changes in depressive symptoms, TCNL used the 16-item Quick Inventory of Depressive Symptomatology (Self-Report) to track the development of her symptoms throughout the intervention. Study administrators did not anticipate the profoundly positive impact that TLNS intervention would have on her depression. In 33 weeks, her depression scores on the QIDS-SR16 decreased from "moderate depression" to "no depression." This was a change of 10 points, which is considered a clinically significant improvement [14-16].

Changes observed in the Emotional subscore of the Stroke Impact Scale (SIS) also contributed to the evaluation of indirect emotional benefits from Translingual Neurostimulation therapy. The Emotional subscore improved 13% within three months, more than two standard deviations above the mean [17]. Furthermore, this level of improvement was maintained for the remainder of the intervention.

Table 2. Indirect Benefits of TLNS Intervention on Speech and Emotion Rehabilitation

Participant 2	QIDS-SR16	DIP	Emotion	Communication	Social Participation	Total Recovery Scale
Baseline	12	123	83.3	71.4	62.5	70
End of Intervention	2	181	94.4	96.4	100	95
Percent Improvement	83.33	48.78	13.33	35.01	60.00	35.71

Table 2 displays scores and percent improvement, from baseline to the end of the intervention, for Subject 2 on measures of speech and emotional rehabilitation. QIDS-SR16 and DIP are separate measurement tools. Emotion, Communication, Social Participation, and Total Recovery Scale are subscores of the Stroke Impact Scale (SIS).

3.2.1. Speech Rehabilitation

Subject 2 also presented with severe dysarthria at the time of enrollment. Speech rehabilitation was monitored through the course of the intervention using the Dysarthria Impact Profile (DIP) [18]. The combination of stimulation and targeted training improved the individual’s performance 58% in the first twelve weeks; a robust change [19]. By the end of the intervention, the subject’s dysarthria recovered nearly 49% from baseline. Congruently, their Communication subscore improved 35% from baseline to a level within one standard deviation of the normal range [17]. The positive changes observed in the participant’s speech are an example of the effectiveness of the TLNS Technology. For the targeted training component of the intervention, subject 2 engaged in specific speech-rehabilitation exercises. Study leaders hypothesize that the degree of her speech recovery, from the chronic stage, may be a consequence of an amplification affect encouraged by the simulation. However, further research is necessary to explore the validity and mechanisms of this proposed interaction.

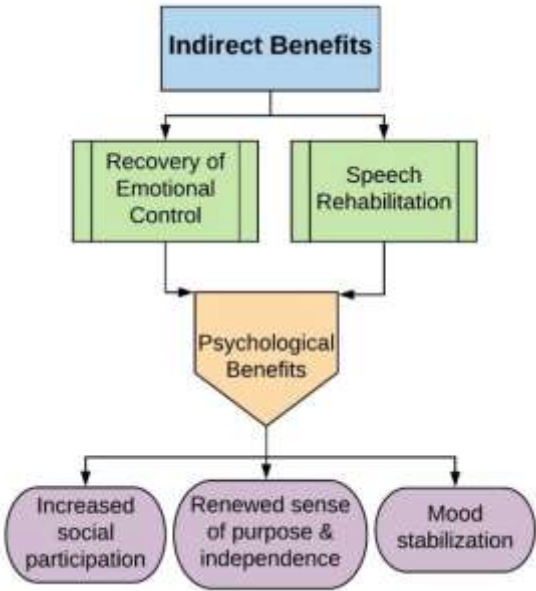


Figure 2: Map of indirect benefits of TLNS Technology gathered from qualitative assessments and participant journals

3.3. Participant Self-Reports

3.3.1. Renewed Quality of Life

Although perception of quality of life (QOL) is highly individualized, it is recognized that an individual's ability to perform Activities of Daily Life/Instrumental Activities of Daily Living (ADL/IADL) may increase their perceived QOL. To explore this line of inquiry, TCNL had subjects record daily journals about their experience and progress throughout the duration of the intervention. The goal of these journals was to gain insight into the potential benefits or losses of QOL related to the intervention. The themes of multiple self-reported improvements are summarized in Figure 3.

As anticipated, subjects' self-reports of improved QOL match the quantified subscores of the SIS test. For example, both subjects reported recovery of participation in social activities, usefulness within their families, and greater independence to perform household activities. Participation/Role Function—a domain of the SIS that would encompass those activities—recovered 60% for one of the participant—an improvement greater than two standard deviations above the mean—and 142% for the other participant (125% of that in the first six months). . These reports are highly encouraging and help to emphasize the importance of this research, specifically for populations who have little to no other options.

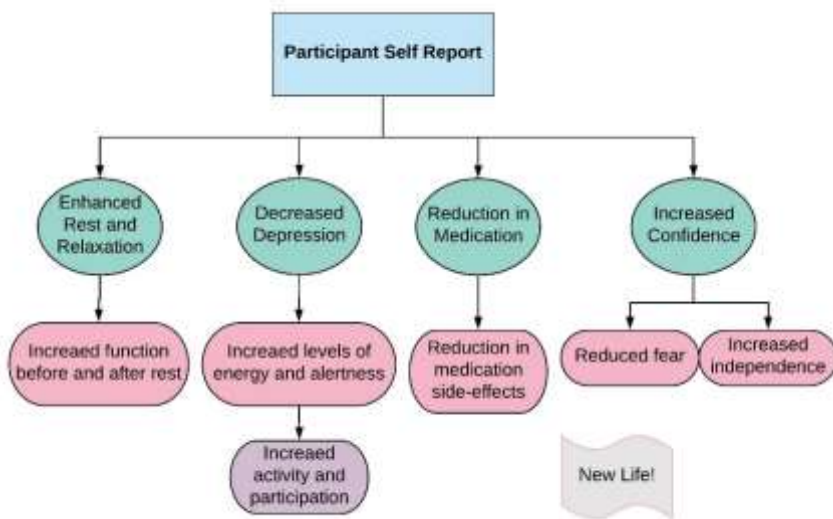


Figure 3 summarizes participant self-reported improvements from TLNS Technology

4. DISCUSSION AND CONCLUSION

Translingual Neurostimulation Technology combines the use of targeted therapy, physical exercise, and non-invasive neurostimulation—delivered directly to the tongue—to enhance natural recovery mechanisms. From this feasibility study we discovered that the effects of the tongue stimulation were more universal effect than expected or previously demonstrated. This study was TCNL's first attempt to provide proof of our concept that *many*

functions can improve with the correct exercise and stimulation. These results provide supporting evidence for major principle of the intervention: that the combination of targeted training and physical exercise with tongue stimulation has the capacity to recover a wide range of symptoms, even from the chronic stage of disease or injury. We entered into a new territory to rehabilitate speech and emotion, and we consider ourselves successful in achieving a positive effect.

The significant finding of this study was that the intervention could be applied to address multiple, diverging symptoms arising from the same disorder (i.e., stroke). The investigators hypothesize that the beneficial effects observed resulted from lasting and cumulative neuroplastic changes (functional, synaptic, and neuronal) in the brainstem and cerebellum on the cellular and neural network levels, elicited by powerful flow of neural impulses (spikes) from the tongue. This feasibility study importantly breaks ground for the scientific community regarding the limits of recoverability and improvement of symptoms for individuals in the chronic stage of their stroke recovery. These findings present a new non-invasive brain stimulation technique with applications in applied and rehabilitative neurosciences. . The results of this feasibility study breaks new ground for the scientific community regarding the limits of recoverability and improvement of symptoms for individuals in the chronic stage of their stroke recovery. Additional research is necessary to understand the potential mechanisms of these phenomena and to optimize efficiency of the intervention.

While it is generally recognized that physical rehabilitation alone has limits for the recovery of chronic symptoms after disease or injury, the limits of combined interventions are more contentious. The idea that TLNS intervention can have a greater or multiplicative effect compared to other forms of intervention (or other forms of combined intervention) remains a question for future research. Next, TCNL intends to compare the impact of TLNS Technology to that of other neurostimulation interventions (such as TMS and TDCS) and to physical interventions alone. The authors leave it to the next generation of investigators to compare the impact of TLNS Technology to that of other neurostimulation interventions (such as TMS and TDCS) and to physical interventions alone.

5. ACKNOWLEDGMENTS

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6. DISCLOSURE

Authors Danilov and Tyler have financial interests in Advanced NeuroRehabilitation LLC and in Helius Medical Technologies, which both have intellectual property rights in the field of use reported in this article.

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Chapter 105

REHABILITATION INTERVENTIONS FOR UPPER LIMB RECOVERY FOLLOWING STROKE

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ABSTRACT

The various treatments are rightly based on the premise that the problems arising following a stroke can be considered recoverable neurological conditions. Spontaneous recovery has its limits and for this reason certain strategies are employed with the aim of stimulating, modulating or controlling this process. Within the rehabilitation process, a fundamental role is played by physiotherapy, the objective of which in the stroke patient is focussed on maximizing the restoration of lost motor functions and re-learning the skills needed for activities of daily living, securing maximum functional capacity and preventing possible secondary complications. Impairment of the upper extremity is one of the most frequent consequences following stroke and it directly impacts on the patient's functioning and quality of life, which justifies the need for a neurorehabilitation treatment appropriate to the specific needs of each patient and based on scientific evidence. In this chapter the different therapeutic strategies used in the field of physiotherapy aimed at the recuperation of the upper limb after stroke are described. Referring to the current literature, the full range of techniques applied in the recovery of these patients is presented, from conventional treatments to emerging approaches and new technologies.

Keywords: stroke, neurorehabilitation, upper limb

INTRODUCTION

Many stroke survivors are left with impairment of the upper limb. Approximately 69% to 80% of these patients initially have upper limb impairments (Kimberley et al., 2004) and

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between 55% and 75% show significant residual deficiencies (Kowalczewski et al., 2007). Patients with hemiplegia after stroke often present impaired motor control and functional disability, such as flaccidity, muscle weakness, abnormal muscle tone, and uncoordinated response, among others (Chen and Shaw, 2006). Functions related to manual dexterity are impaired in a high percentage of patients between 3 and 6 months after the illness, and complete functional recovery of the hand has only been documented in between 5% and 20% of cases (Alon et al., 2007). Therefore, numerous rehabilitative strategies have been proposed in order to improve recovery in stroke patients as soon as possible.

The various treatments are rightly based on the premise that the problems arising following a stroke can be considered recoverable neurological conditions (Butefisch, 2004; Carr and Shepherd, 1989). This process of recuperation will depend on various factors such as the age of the patient, hemispheric dominance, premorbid intellectual level, time since the appearance of the lesion and its size, extent and aetiology, among other factors (Díaz et al., 2011). Spontaneous recovery has its limits and for this reason certain strategies are employed with the aim of stimulating, modulating or controlling this process (Bayón and Martínez, 2008). Within the rehabilitation process, a fundamental role is played by physiotherapy, the objective of which in the stroke patient is focussed on maximizing the restoration of lost motor functions and re-learning the skills needed for activities of daily living (ADL) (Cameirão et al., 2009), securing maximum functional capacity and preventing possible secondary complications (Stokes, 2001).

As Pollock et al. (2008) observe in their meta-analysis, a range of different post-stroke strategies exist, although the difficulty of defining specific therapeutic methods, the lack of appropriate measurements of the results, the multitude of variables which participate in the treatment process and the heterogeneity of the sample characteristics have presented problems in achieving a meaningful evaluation of the different treatments. The best strategies for the recovery of the upper limb are still unknown (Chen and Shaw, 2006; Yozbatiran et al., 2006), for despite the routine use in clinical practice of traditional methods like those described below, there is still insufficient evidence about these treatment approaches. Nor is there any evidence available to date that proves that any one physiotherapeutic method is better than any another (Kollen et al., 2009; Barreca et al., 2003). Nevertheless, certain studies have indicated that physiotherapy as a whole can be beneficial (Kwakkel et al., 1999; Feys et al., 1998). Specifically, early intervention can be better than late (Cifu et al., 1999), but even treatments applied belatedly result in benefits (Farmer et al., 2014).

The optimum treatment characteristics are unknown (Farmer et al., 2014), but reference has been made to the fact that elements such as intensive activity, repeated practice, task-oriented activity, active participation of the subject and cognitive stimulation are crucial in achieving results (Veerbeek et al., 2014; Timmermans et al., 2009; Bayón and Martínez, 2008; Alon et al., 2007; Chen and Shaw, 2006; Pomeroy and Tallis, 2002). The heterogeneity of the treatment parameters and the characteristics of the population make generalized recommendations difficult, so further investigation is needed in order to define more precisely criteria related to the population, the treatment, the comparison with other interventions and their results (Farmer et al., 2014). The evidence base for stroke rehabilitation continues to grow; this includes studies using neuro-imaging as well as studies of novel therapies which might influence stroke rehabilitation practice and policy in the future (IJzerman et al., 2009). Referring to the currently available literature, various physiotherapeutic treatments used to promote upper limb recovery in patients following stroke will now be detailed.

CONVENTIONAL NEUROLOGICAL TREATMENT

The main traditional physiotherapeutic methods used to improve impairment of the affected limb and to recover motor function for stroke patients have been based on neurophysiological theory or motor relearning (Chen and Shaw, 2006). This conventional rehabilitation mainly consists of the Bobath approach, the proprioceptive neuromuscular facilitation technique, the Brunnstrom approach and the motor relearning technique (Pollock et al., 2014; Oujamaa et al., 2009; Chen and Shaw, 2006; Pomeroy and Tallis, 2002; Sparkes, 2000).

The Bobath concept, also known as neurodevelopmental treatment (NDT), is the most popular technique for stroke rehabilitation (Chen and Shaw, 2006). This method seeks to normalize tone through techniques of inhibition and facilitation of correct movement by means of manipulation of the key points. Many authors have identified the problem of a lack of published theoretical evidence to support the Bobath concept (Sparkes, 2000). It has been pointed out that some physiotherapy given within a Bobath framework might have an immediate beneficial effect on conduction in the corticospinal pathway and the ability to control joint movement (Pomeroy and Tallis, 2002).

The proprioceptive neuromuscular facilitation technique employs techniques to facilitate normal patterns of movement, spirals and diagonals, with the objective of inducing generalized patterns and thus generating a balance between agonists and antagonists, which permits both postural and motor control.

The Brunnstrom approach is based on observations of movement recovery in a vast number of stroke patients. Brunnstrom identified six different motor recovery stages after stroke; each grade was based on the degree of motor ability of the affected limb (Chen and Shaw, 2006). This method employs associated reactions, tonic reflexes and the development of basic synergistic movements to facilitate normal movement.

Finally, the motor relearning technique described by Carr and Shepherd incorporates principles based on specific training in tasks, including the practice in isolation of impaired essential movements and their subsequent training in functional tasks and activities. The Bobath theory, aiming at restoring postural control as a prerequisite to repetitive task training in order to be as physiological as possible, is opposed to theories inspired by these authors on motor skill training where the training programme should focus on performing a functional task, regardless of the motor strategies used (Oujamaa et al., 2009).

The body of evidence in systematic reviews and meta-analyses shows that when comparing improvement of upper-limb motor control in response to therapy, the Bobath approach seems to be no more effective than the rest of the strategies named (Chen and Shaw, 2006). The same point is also made by Barreca et al. (2003), who add that NDT was not found to be superior to other types of interventions such as forced use and traditional rehabilitation functional training.

It has also been reported that these approaches are less effective when compared to focused interventions such as modified Constraint-Induced Movement Therapy (CIMT), bilateral arm training or strengthening when applied in a task-specific way (Veerbeek et al., 2014). In the same vein, Hatem et al. (2016) concluded that there is moderate to high-quality evidence indicating that Bobath therapy is similar or inferior to other rehabilitation approaches such as meaningful task-specific training, CIMT, ARM-basis training, motor

relearning programmes and movement science-based physiotherapy for treating upper limb motor impairment and disabilities in acute, subacute and chronic stroke patients. On the other hand, it should be pointed out that one moderate-quality randomized controlled trial (RCT) was reviewed which indicated that Bobath therapy might be useful in patients with spasticity (Wang et al., 2005).

Veerbeek et al. (2014) in their exhaustive meta-analysis dealing with NDT found strong evidence for equal effectiveness compared to other interventions for muscle strength of the arm and insufficient evidence for grip strength, muscle tone, brain activity, and extended ADL. Furthermore, the effect of more time spent in NDT was also investigated, indicating that NDT is equally as effective as augmented NDT for muscle strength, arm-hand activities, basic ADL, extended ADL, pain, handicap, and quality of life outcomes; and augmented NDT is beneficial for motor function and range of motion.

KINESITHERAPY

On many occasions kinesitherapy also plays a part in conventional physiotherapeutic protocols. It involves joint mobilization, strength training, stretching, hands-on therapy and specific exercises utilizing objects and equipment (Farmer et al., 2014; Veerbeek et al., 2014; Pollock et al., 2014).

Therapists use a variety of techniques to promote upper limb recovery following stroke; however, the details of what they do are unclear, are not well defined in studies, and they are used in various combinations (Winter et al., 2011). The small number of trials and the methodological limitations make it inappropriate to draw conclusions from the effectiveness of hands-on therapy techniques (Pollock et al., 2014; Winter et al., 2011). The limited evidence of benefits resulting from stretching, passive exercises and mobilization, when applied to the hemiplegic upper limb following stroke, merits further research (Winter et al., 2011).

Concerning stretching, Hatem et al. (2016) argued that there is moderate to high-quality evidence indicating that stretching is similar to control rehabilitation approaches for treating upper limb impairments and disabilities in subacute and chronic stroke.

The findings from the meta-analysis carried out by Harris and Eng (2010) provide evidence that strength training can improve function without increasing tone or pain in stroke patients. A positive outcome for strength training was found for grip strength and upper-limb function and no benefit in ADL. Furthermore, a significant effect for strength training on upper-limb function was found for trials including subjects with moderate and mild upper-limb motor impairment. The authors recommend that future RCTs investigate the intensity, frequency, and specificity of strength training required for improving performance in daily activities. Ada et al. (2006) also provide support for the use of strengthening training as a part of rehabilitation programmes after stroke. In contrast, the meta-analysis by Veerbeek et al. (2014) showed non-significant effect sizes for motor function, muscle strength, range of motion and pain in the case of strength exercises for the paretic arm.

Isokinetic muscle strengthening is another rehabilitation technique, although this is not often used in stroke patients. It uses computer-driven isokinetic dynamometers which allow training for muscle strength (Hatem et al., 2016). The review presented by Hammami et al.

(2012) included two studies on this kind of treatment related to upper extremity following stroke. There is no consensus on the type of contraction mode, on the muscles that should be trained and on the dosage regimen of training, and there are no studies that have investigated the effects of isokinetic strengthening of wrist and finger muscles. There is not sufficient evidence to claim the superiority of this technique over conventional strengthening exercises (Hattem et al., 2016). More RCTs are needed in order to assess their effectiveness especially concerning upper arm rehabilitation (Hammami et al., 2012).

THERAPEUTIC POSITIONING OF THE PARETIC ARM

Also described in the literature are techniques focused on the positioning of the paretic arm, either without the use of external devices or its immobilization or stabilization by means of splints, support by weights, bandages or other technical aids, with the aim of maintaining or augmenting the range of movement, preventing incorrect positions, inhibiting reflexes, reducing muscle tone and also reducing oedema and pain (Veerbeek et al., 2014; Pollock et al., 2014; Langhorne et al., 2009; Barreca et al., 2003).

Therapeutic positioning of the paretic arm, without the use of splints, with the purpose of maintaining range of motion and preventing harmful positions of the paretic arm was investigated by Veerbeek et al. (2014). A significant positive effect size was found for passive range of motion of shoulder external rotation and non-significant effect sizes were found for passive range of motion of shoulder internal rotation, external rotation contracture of the shoulder, pain at rest and while moving, and basic ADL.

Regarding shoulder supports, low-quality evidence derived from an out-of-date review (Ada et al., 2005) shows no benefit of shoulder-supporting devices on arm function, shoulder external rotation and contracture.

Borisova and Bohannon (2009) investigated the effectiveness of the positioning of the paretic shoulder following stroke. This meta-analysis failed to support the benefit of using these techniques in order to prevent or reduce shoulder external rotation range of motion impairments after stroke. The authors stated that future trials should assess the effectiveness of longer duration positioning and of intervention earlier in the recovery process. The effect of positioning on other shoulder movements and on other joints should also be investigated, as should the effect of positioning and the combination with other treatments.

There is insufficient evidence to support or refute the effectiveness of elbow orthoses and hand splinting (Pollock et al., 2014; Hijmans et al., 2004; Lanning and Herbert, 2003). Further research is required in this field for evidence-based prescriptions. Lanning and Herbert (2003) suggested that future RCTs should not only consider the effects of different splint designs but also consider the effects of the time period for which a splint is worn and the hand's position within the splint.

An up-to-date meta-analysis (Veerbeek et al., 2014) pointed out that the use of reflex-inhibiting positions or local immobilization of the wrist and hand by splints or plaster have no significant effect for passive range of motion, muscle tone and pain. These authors also added that supportive techniques or devices for the prevention or treatment of glenohumeral subluxation or hemiplegic shoulder pain did not show significant effects for motor function of the paretic arm or for pain.

BILATERAL ARM TRAINING

Bilateral arm training is another rehabilitation technique suggested for upper limb recovery following stroke (Pollock et al., 2014; Veerbeek et al., 2014; Timmermans et al., 2009; Oujamaa et al., 2009; Urton et al., 2007). During bilateral arm training, movement patterns or activities are performed with both hands simultaneously but independent from each other and can be cyclic (Verbeek et al., 2014). It can be performed with or without the assistance of an external device (Hattem et al., 2016).

Bilateral movement training validated by the meta-analysis of Stewart et al. (2006) does not appear clearly superior or even as efficient as the unilateral mode in some other studies. Various reasons could explain these divergent results, such as post-stroke delay, degree of motor impairment, type of bilateral movement training proposed and amount of movement repetitions (Oujamaa et al., 2009). The key point of this kind of intervention is interlimb coupling, which is thought to rebalance interhemispheric inhibition, activate the affected hemisphere, and improve motor control within the affected limb (Pollock et al., 2014), although at present the neurophysiological processes that support this rehabilitation approach are still not fully understood.

Coupar et al. (2010) concluded that there is insufficient good quality evidence to make recommendations about the effect of simultaneous bilateral training compared to placebo, no intervention or usual care, for improving arm function after stroke. On the other hand, van Delden et al. (2012) compared the effects of unilateral and bilateral training and they claim that unilateral and bilateral training are similarly effective; however, intervention success may depend on severity of upper limb paresis and time of intervention post-stroke.

Considering more recent evidence, the meta-analyses presented by Veerbeek et al. (2014) showed non-significant effect sizes for motor function of the paretic arm, muscle strength, arm-hand activities, self reported arm-hand use in daily life, and basic ADL. In the same vein, the systematic review by Hattem et al. (2016) furnished moderate to high-quality evidence that bilateral arm training is similar or inferior to unilateral arm training or to conventional treatment. These authors also argued that its effectiveness on spasticity is also demonstrated to be controversial, owing to the disparity in results.

BIOFEEDBACK

Biofeedback provides enhanced awareness of movement or function, with the goal of improving voluntary control of that movement or function. It can be associated with electromyography (EMG) which provides information about muscle activity detected through surface electrodes placed on the skin, and it is fed back to the patient via electrical activity displayed on a visual display unit or by an auditory signal (Pollock et al., 2014). This technique has also been used in upper limb recovery after stroke (Pollock et al., 2014; Brewer et al., 2013; Timmermans et al., 2009; Langhorne et al., 2009; Chen and Shaw, 2006; Barreca et al., 2003).

The use of EMG biofeedback was previously endorsed by Hiroaka (2001), who stated that EMG biofeedback has a large effect on restoring arm function in post-stroke patients. In relation to this, Molier et al. (2010) pointed out that feedback has an added value for stroke

rehabilitation, although it was not possible to find which factors and types of feedback are most necessary for a beneficial effect on motor activities and motor functions, due to the combination of multiple aspects and types of feedback in the reviewed studies. With regard to stroke phase, a recent meta-analysis (Wattchow et al., 2017) that investigated therapeutic interventions in improving upper limb function in the first 4 weeks following stroke, has recommended the supplementary use of biofeedback within the acute phase post-stroke.

In contrast, the meta-analyses by Veerbeek et al. (2014) on this topic resulted in non-significant effect sizes for motor function of the paretic arm, active range of motion, and arm-hand activities; and Woodford et al. (2007) pointed out the existence of low-quality evidence when EMG biofeedback is compared with standard physiotherapy.

SENSORY INTERVENTIONS

Other therapeutic strategies in the treatment of the upper limb include sensory interventions (Farmer et al., 2014; Veerbeek et al., 2014; Pollock et al., 2014). Movement and somatosensory awareness can be enhanced in several ways, including techniques such as sensory re-education, tactile kinaesthetic guiding, repetitive sensory practice or desensitisation. Sensory and positional awareness may be stimulated by passive or active-assisted movement, as well as by stimulatory techniques such as stroking and tapping (Pollock et al., 2014).

Different interventions or upper limb sensory impairment after stroke are available but there is insufficient evidence to support or refute their effectiveness in improving sensory impairment, upper limb function, or participants' functional status and participation (Doyle et al., 2010). Schabrun and Hillier (2009) demonstrated some support for the effectiveness of passive sensory training in relation to sensory impairment and motor function. However, empirical evidence for active sensory training is limited. The scarcity of good quality evidence available on the variety of sensory interventions and the clinical and methodological diversity between the studies requires higher quality research, where more well-designed, better reported studies of sensory rehabilitation are needed (Doyle et al., 2010; Schabrun and Hillier, 2009).

The analyses of one recent meta-analysis (Verbeek et al., 2014) showed significant positive effect sizes for somatosensory functions and muscle tone, but in contrast, it resulted in non-significant effect sizes for motor function of the paretic arm, muscle strength, pain, arm-hand activities, and basic ADL.

COGNITIVE SENSORY MOTOR TRAINING THERAPY

Among the various therapeutic interventions, it is also worth noting the Cognitive Therapeutic Exercise based on the neurocognitive rehabilitation theory described by Perfetti (1999). This method is based on an approach which uses cognitive strategies such as observation of actions, the motor imagery or imitation in therapeutic exercises, in conjunction with guided activation of attention or problem solving for the recovery of movement after stroke (Sallés, 2015).

Currently there is very little evidence available on this method (Hatem et al., 2016). Wongphaet et al. (2003) carried out a report of case studies concluding that cognitive sensory motor training therapy may be an effective method for improving upper extremity motor function in chronic stroke and patients, but further RCTs are required. On the other hand, the study by Chanudol et al. (2012) showed no difference between Perfetti's method and conventional occupational therapy with regards to hand and arm function after a stroke. Lee et al. (2015) claimed that application of cognitive exercise therapy was effective for upper limb function and quality of life in chronic stroke patients. Future research should focus on application of cognitive exercise therapy in diverse populations, and assess its clinical utilization. The most recent study, published by Sallés et al. (2017), argued that despite the lack of statistical significance, upper extremity movement deficits improved when this neurocognitive approach was performed. The authors also stated that a careful selection of the appropriate difficulty for each patient together with the guidance of a therapist in the cognitive and sensory processes allowed greater and more prolonged improvements, especially for the hand function. Finally, the feasibility of the proposed protocol facilitates further research to consolidate evidence of these findings.

PHYSICAL AGENTS

Reference must also be made to interventions with physical agents such as electrical current, both in the form of TENS (Transcutaneous Electrical Nerve Stimulation) with analgesic aims or for somatosensorial stimulation at the sensitive threshold, and also in its neuromuscular application stimulating a muscular contraction (Veerbeek et al., 2014; Pollock et al., 2014; Hayward et al., 2010; Oujamaa et al., 2009; Langhorne et al., 2009; Urton et al., 2007; Chen and Shaw, 2006; Barreca et al., 2003); and also the use of thermal agents (Veerbeek et al., 2014; Oujamaa et al., 2009; Chen and Shaw, 2006) like cold or heat.

In the first approach related to *electrotherapy*, the stimulation evokes a sensory reaction without muscle contraction. According to Hatem et al.'s review (2016) regarding sensory electrical stimulation, there is moderate-quality evidence that TENS as an adjuvant to rehabilitation treatment is superior to the rehabilitation treatment alone with regards to upper extremity impairments and disabilities. Spasticity seems to diminish with this technique. Moreover, TENS appears to be beneficial in the subacute and chronic post-stroke phase.

In the second approach, where a muscular contraction is provoked, known as neuromuscular electrical stimulation (NMES), several applications can be distinguished: cyclic NMES (without voluntary activity by the patient), sensor or electromyographic (EMG)-triggered NMES (activated voluntarily by the patient) and functional electrical stimulation (FES) (associated with the performance of functional tasks) (Sentandreu, 2017). In some of these cases, NMES treatment can be applied by orthosis

A few previous studies have directly compared electrical stimulation modalities, yet conclusive proof of their effects is still lacking (Hara, 2008). De Kroon and IJzerman (2008) and Boyaci et al. (2013) compared cyclic NMES and EMG-triggered NMES and no difference in outcomes was found. These findings contrast with other reviews (Chen and Shaw, 2006; de Kroon et al., 2005), which argued that electrical stimulation triggered by voluntary movement appear to be more beneficial than other NMES modes. It has been

hypothesized that the intention to execute a movement, along with peripheral activity, further reinforces plastic changes compared to cyclic stimulation (Quandt and Hummel, 2014). A recently published study (Wilson et al., 2016) that compared the effect of cyclic NMES, EMG-triggered NMES and sensory stimulation, concluded that all groups exhibited significant improvement of impairment and functional limitation in the upper limb, though there was no difference based on the type of electrical stimulation administered.

Different authors support the use of NMES (Wattchow et al., 2017; Hatem et al., 2016; Howlett et al., 2015; Nascimento et al., 2014) in the recovery of stroke patients. However, there are various points that are inconsistent. The most recent research on electrical stimulation and upper limb recovery focuses mainly on the most effective protocols by comparing different variables of the applied programmes (i.e., NMES modalities, stimulation parameters, electrode placement, etc.), the characteristics of the patients who can benefit most from this treatment, its effectiveness as a complement to other emerging therapies, and not only its effects locally but also its impact on the central nervous system (Sentandreu, 2017). Hatem et al. (2016) claimed that there was moderate-quality evidence that NMES in combination with rehabilitation is superior to the rehabilitation treatment alone with regards to upper extremity impairment. It seems not to be effective on upper extremity disabilities. The review by Eraifej et al. (2017) found a statistically significant benefit from FES applied within 2 months of stroke on the primary outcome of ADL. The supplementary use of electrical stimulation within the acute phase post-stroke also was supported by Wattchow et al. (2017).

On the contrary, there are authors who indicate that its effectiveness is not clear. Pollock et al. (2014) indicated low-quality evidence related to the effectiveness of electrical stimulation. For their meta-analyses, Veerbeek et al. (2014) divided electrostimulation into NMES, EMG-triggered NMES, and TENS, and they only found significant effects on the use of wrist and finger flexors and extensors for NMES, shoulder muscles for NMES and wrist and finger extensors for EMG-triggered NMES. The other modalities analyzed did not show differences. Some authors (Gu and Ran, 2016; Vadafar et al., 2015) pointed out that electrical stimulation could be used in order to prevent or reduce shoulder subluxation early after a stroke, yet findings did not support their efficacy for pain reduction, improvement in arm strength, movement, functional activity, and quality of life following a stroke.

In the case of *thermal agents*, further research is needed to establish a basis for this type of treatment and to permit the making of prescriptions based on evidence. The study by Chen et al. (2005) demonstrated significant effects on several aspects of sensory and motor functional improvement in acute stroke with lower motor stage or flaccid motor control of the upper limb. The authors posed a number of questions that should be clarified in the future such as the optimal intensity, the time course of thermal stimulation, and also the patients appropriate for this type of treatment (Chen and Shaw, 2006).

EMERGING APPROACHES

Among the emerging approaches, that are based on a supposed cortical reorganization and optimization of motor relearning, are included repetitive and task-specific training, CIMT and other interventions based on the hypothesis of mirror neurons and motor imagery.

Repetitive task training involves the repeated practice of functional tasks, combining elements of intensity of practice and functional relevance (French et al., 2016). It is a component of current physiotherapy approaches in stroke rehabilitation (Pollock et al., 2014; Timmermans et al., 2009; Langhorne et al., 2009; Barreca et al., 2003). Key components of skill acquisition, such as active cognitive involvement, functional relevance of the task and knowledge of results and performance, are hypothesised to enhance learning during repetitive task training (Pollock et al., 2014). On the other hand, *task-specific training*, also referred to as functional task training, involves practice of tasks relevant to daily life and it is often combined with other modalities, such as assistive technologies (Timmermans et al., 2009). Task-specific training may be carried out as a form of repetitive task training (Pollock et al., 2014).

A recent Cochrane review (French et al., 2016) pointed out that there is low-quality evidence of improvement in arm and hand function following repetitive task training of the upper limb, and effects appear to be sustained up to 6 months post treatment. The authors also added that adverse effects should be monitored if it is put into practice and that more intensive therapy (over 20 hours) does not seem to be more effective for the upper limb. The authors' recommendations highlight that future research should evaluate practical ways of delivering repetitive task training interventions and maintaining post-therapy functional gain beyond 6 months; and these trials also should check clinical effect and cost-effectiveness analysis, in addition to including quality of life outcomes.

In an updated version of the last-named review, Thomas et al. (2017) concluded that patients who receive repetitive task training may be more likely to improve upper limb function after treatment and maintain these improvements during the 6 months after treatment than patients receiving usual care. The authors' findings indicate that patients appear to benefit from repetitive task training regardless of the amount of task practice, type of intervention, or time since stroke. Further RCTs should focus on the type and amount of training, including ways of measuring the number of repetitions actually performed by subjects.

With regard to task-specific training, evidence was insufficient to obtain conclusions related to the effectiveness of reach-to-grasp exercise-related interventions (Pelton et al., 2012). The authors stated that what is needed is RCTs with sufficient power and standardised outcome measures for future meta-analysis that should detail functional performance and kinematic measures of hand and arm coordination. In a recent meta-analysis by Wattchow et al. (2017) where rehabilitation interventions for upper limb function within the acute phase post-stroke were studied, key findings included significant positive effects for task-specific training in the first 4 weeks following stroke.

CIMT as described by Taub et al. (2006) is the most complete application of the functional task paradigm that applies motor skill learning principles to stroke rehabilitation. Its specific strategy is to induce motor learning and neuroplasticity with intensive training intervals (Hattem et al., 2016). CIMT differs from forced-use in that it requires both functional training of the affected arm and immobilization of the patient's non-affected upper extremity. The original high-intensity protocol of CIMT is well defined, although later modified CIMT protocols have been described with lower dosage regimens. This therapy seems to have a significant effect on upper-limb recovery; however, the application is limited to stroke survivors who have some distal voluntary movement in the upper limb (Chen and Shaw, 2006), are highly motivated, free of severe cognitive disorders and not at risk of falling

(Oujamaa et al., 2009). The main challenge in applying the evidence of benefit from CIMIT is that the intervention is time-consuming and tiring, presenting major challenges to compliance (Langhorne et al., 2009).

Hatem et al. (2016) showed moderate-quality evidence that CIMIT is superior to conventional rehabilitation with regards to upper extremity impairments and disabilities. This intervention seems to be effective in the different phases of stroke and its effects may persist till 12 months after training. According to the authors, CIMIT, either in its original or modified design, can be recommended for stroke patients after 3 months and in patients with active hand movement. Pollock et al. (2014) also indicated moderate-quality evidence of CIMIT effectiveness on measures of upper limb function. Key findings by a recent meta-analysis (Wattchow et al., 2017) included significant positive effects for modified CIMIT within the acute phase post-stroke. According to Veerbeek et al. (2014), significant positive effect sizes were found for arm-hand activities, self-reported amount of arm-hand use in daily life, and self-reported quality of arm-hand movement in daily life. Due to the size of the study sample and the low risk of bias, this result is classified as level 1 evidence, when referring to the original CIMIT treatment. Finally, a recent meta-analysis by Liu et al. (2017) also demonstrates that CIMIT and modified CIMIT might be more beneficial than conventional rehabilitation in the acute and subacute post-stroke phase.

Mirror therapy is a rehabilitation approach for stroke patients with upper extremity motor impairment (Veerbeek et al., 2014; Pollock et al., 2014; Brewer et al., 2013; Oujamaa et al., 2009) which is subtended by the hypothesis of the mirror neuron system, which discharges both on the occasion of performing a motor act or of simply observing it being performed by another subject (Hatem et al., 2016). This treatment consists of presenting visual feedback using a mirror placed in the midsagittal plane of the patient and reflecting the non-paretic side as if it was the affected one. Using this arrangement, the patient should be placed so that s/he sees only the reflection of the non-paretic upper limb in the mirror, creating for the patient the visual illusion of normal movements of the paretic limb (Pérez-Cruzado et al., 2017; Hatem et al., 2016).

Despite being a technique that emerged for the treatment of amputees' phantom limb pain and that has recently been used for patients after stroke, it has enough evidence to support its use in upper extremity recovery following stroke. Accordingly, the findings by Thieme et al. (2012) indicate evidence for the effectiveness of mirror therapy for improving upper extremity motor function, ADL and pain, at least as an adjunct to conventional rehabilitation for patients after stroke. Hatem et al. (2016) pointed out that there is moderate-quality evidence that mirror therapy is superior to sham therapy, control therapy or standard rehabilitation treatment with regards to upper extremity impairments and disabilities. Mirror therapy effects may persist till 6 months after treatment and it appears to be effective in the different phases of stroke. Pérez-Cruzado et al. (2017) confirmed that the combination of mirror therapy with conventional treatment showed greater effectiveness in the treatment of upper limb function in both acute and chronic stroke survivors. The authors added that for maximum effect, mirror therapy interventions should consist of 20-minute sessions delivered 5 days per week for 4 weeks. Finally, a recent meta-analysis by Zeng et al. (2018) also provided some evidence that mirror therapy may significantly improve motor function of the upper limb in patients with stroke, although the author insisted on the importance of carrying out studies to investigate this technique in more depth.

Mental practice with motor imagery is another emerging approach proposed for stroke patients (Veerbeek et al., 2014; Pollock et al., 2014; Oujamaa et al., 2009; Langhorne et al., 2009; Zimmermann-Schlatter et al., 2008). Mental imagery can be defined as the conscious representation of an action and is based on a subliminal activation of the motor neuron system. This approach is not only involved in performing a movement but also in imagining actions, recognizing various tools, comprehending another person's behaviour and observational learning (Oujamaa et al., 2009). The protocols used vary from one study to another, where the mental imagery exercises can be performed in the first (as an actor) and third person (as a spectator).

At present, mental practice with motor imagery seems to be valuable in improving upper extremity recovery in post-stroke patients. Different reviews and meta-analyses support its use. Hatem et al. (2016) showed moderate-quality evidence that mental practice in combination with another rehabilitation treatment is superior to the other rehabilitation treatment alone with regards to upper extremity impairments and disabilities and it appears to be effective in both the subacute and chronic phase. A Cochrane systematic review by Pollock et al. (2014) showed moderate-quality evidence indicating a beneficial effect of mental practice performed in addition to conventional exercise-based interventions on arm function and impairment. In contrast, Veerbeek et al. (2014) investigated mental practice combined with physical practice including RCTs with acute and chronic post-stroke patients, and they showed a significant positive effect size for arm-hand activities but non-significant effect sizes for motor function of the paretic arm, muscle strength, and basic ADL.

As to future lines of research, studies should be directed to a more specific cognitive assessment in order to differentiate the subjects capable of correctly performing mental imagery exercises from those who are unable to perform them correctly, and should also study in greater depth populations in the subacute post-stroke phase and with severe motor impairment (Oujamaa et al., 2009).

TECHNOLOGY SUPPORTED TRAINING

There has been a great advance in rehabilitation technology in the last decade that has created a large variety of new opportunities for patients and therapists (Timmermans et al., 2009). Robotics, virtual reality, and brain-machine interfaces, among others, are playing leading roles in this innovation process.

Virtual reality involves interactive simulations created by computer to provide a simulated practice environment, as well as feedback on movement execution or goal attainment (Pollock et al., 2014). Several virtual reality systems have been developed for motor rehabilitation of the upper extremity following stroke based on different paradigms and hypotheses such as learning by imitation, reinforced feedback, haptic feedback, augmented practice and repetition, action execution-observation, and others (Cameirão et al., 2008).

Currently, the most used virtual reality systems in rehabilitation processes are the IREX system, Rutgers Master II-ND and CyberGlove. Most trials conducted with virtual reality systems include a limited number of patients in the subacute or chronic post-stroke phase, with the presence of distal residual active movement and without previous cognitive deterioration and severe balance disorders (Bayón and Martínez, 2008).

Virtual reality training for the paretic arm resulted in a significant positive effect size for basic ADL, according to the result of the meta-analysis by Veerbeek et al. (2014). In contrast, a Cochrane review (Laver et al., 2011) investigating the use of virtual reality and interactive video gaming in stroke rehabilitation reported that there was limited evidence of any benefit in improving arm function and ADL when compared with the same dose of conventional therapy.

After analyzing the data, Hatem et al. (2016) showed that there is moderate-quality evidence that virtual reality is similar to standard intervention with regards to upper extremity impairment and disabilities and that this technique combined with another rehabilitation treatment is superior to the other rehabilitation treatment alone with regards to upper extremity impairment and activities. Concerning virtual reality immersion, there is moderate-quality evidence that it is superior to standard treatment with regards to upper extremity impairment and disabilities.

Virtual reality gaming has emerged with commercial gaming consoles in particular, being rapidly adopted in clinical settings (Laver et al., 2017). The literature supports the use of this rehabilitation therapy as equivalent to traditional therapies or as successful augmentation of those therapies (Yates et al., 2016). Hatem et al. (2016) showed moderate-quality evidence indicating its superiority to standard rehabilitation treatment with regards to upper extremity impairment.

Although individual trials have reported benefits, future studies should incorporate larger sample sizes and post-intervention follow-up (Yates et al., 2016), should demonstrate measures of efficacy for a particular type or degree of impairment (Dobkin et al., 2013) as well as developing new portable and low cost virtual reality systems that allow accessibility to this type of treatment even in the home setting (Bayón and Martínez, 2008). Regarding other more technical requirements, Oujamaa et al. (2009) also remarked that technological advances are expected to reduce the kinetosis linked to the time delay between the visual information received by the subjects and their movements performed in total immersion.

An up-to-date Cochrane review (Laver et al., 2017) found evidence that the use of virtual reality and interactive video gaming was not more beneficial than conventional therapy in improving upper limb function. Virtual reality may be beneficial in improving upper limb function and ADL function when it is used as an adjuvant to standard care. The authors also pointed out that time since onset of stroke, severity of impairment, and the type of device were not strong influencers of outcome. Furthermore, it was suggested that higher dose and customised virtual reality programmes were preferable.

Robotic-assisted treatment is another therapy that has been advocated to improve upper limb recovery (Veerbeek et al., 2014; Pollock et al., 2014; Brewer et al., 2013; Hayward et al., 2010). Robotic devices can move passive limbs, while providing assistance or resistance to movement of a single joint or control of intersegmental co-ordination (Mehrholtz et al., 2012). Currently, there are different systems named in the literature such as NeReBot, InMotion2 unimanual robot, Bi-Manu-Track robot, MIME robot, BATRAC, MIT-Manus robot, Myomo e100, and others (Oujamaa et al., 2009; Bayón and Martínez, 2008). The main advantages of using robot-assisted therapy are to deliver high-dosage, repetitive, interactive and high-intensity training (Veerbeek et al., 2014; Sivan et al., 2011; Bayón and Martínez, 2008). Most robotic devices are usually tailored for elbow and shoulder movements (Hatem et al., 2016). Such therapy can be used individually or combined with other therapeutic interventions such as the use of FES. The integrated use of FES and robotic devices is called

hybrid robotic rehabilitation and it has emerged as a promising approach for rehabilitation of upper limb motor functions (Resquín et al., 2016).

Meta-analyses by Veerbeek et al. (2014) resulted in significant positive effect sizes for motor function of the proximal part of the paretic arm, muscle strength, and pain with regard to shoulder-elbow robot-assisted therapy and in significant positive effect sizes for motor function of the paretic arm and muscle strength with regard to elbow-wrist robotics. A Cochrane review (Mehrholtz et al., 2012) reported that patients who receive electromechanical and robot-assisted arm training after stroke are more likely to improve their arm motor function and generic ADL. A recent systematic review with a meta-analysis of RCTs (Zhang et al., 2017) was carried out to evaluate the effectiveness of robotic training and conventional training in improving the motor recovery of paretic upper limbs in stroke patients. The authors concluded that this intervention appeared to have positive outcomes in enhancing motor recovery of the paretic upper extremity and they support robot-assisted therapy in addition to conventional treatment in order to help both therapists and patients in the management of upper limb recovery. In contrast, Hatem et al. (2016) observed that there was moderate-quality evidence that this therapy for the paretic upper limb is similar or inferior to standard treatment and, in addition, any gains obtained are specific to the task that is being practised and do not extrapolate to ADL. In the same way as the last-named author, Norouzi-Gheidari et al. (2012) demonstrated evidence of no benefit or harm of robotics, compared with the same duration of conventional rehabilitation.

Although there is evidence to suggest a potential future role for robotic-assisted therapy in stroke rehabilitation, the results must be interpreted with caution because there are variations between the trials in the duration and amount of training, type of treatment, and the patient characteristics (Mehrholtz et al., 2012) and moreover, there is much left to explore in this area (Brewer et al., 2013). Future large studies should determine the cost-benefit and clinical effectiveness of robotic therapies in stroke rehabilitation. It is still necessary to design robots that can re-educate more complex motor functions and establish therapeutic protocols that are cost-effective. As more studies are available that include a greater number of patients, with different degrees of motor impairment, further advances will be made in determining the efficacy and advantages of this therapy, which may contribute to the extension of its application in daily clinical practice (Bayón and Martínez, 2008).

Recent advances in neurotechnology have led to the development of *brain-computer interfaces* (BCIs) that translate brain activity into control signals of computers (Venkatakrishnan et al., 2014). These innovative technologies provide a means to decode mental states and activate devices according to user intentions, and could provide direct feedback on the engagement of motor areas of the brain surrounding the lesion site (Millán et al., 2010). BCI technology potentially promotes neuroplasticity and Hebbian-based motor recovery by rewarding cortical activity associated with sensory-motor rhythms through use with a variety of self-guided and assistive modalities (Remsik et al., 2016), yet a better understanding of mechanisms underlying brain recovery is required in order to improve efficacy of BCIs in stroke neurorehabilitation. As has already been mentioned, such intervention may be used individually, integrated with other therapeutic modalities or even combined with output devices such as FES (Silvoni et al., 2011). BCI with a FES system has been explored as a potential rehabilitation tool for motor learning after stroke because this combined system can assist such patients in learning and practicing the desired coordinated movements (Biasiucci et al., 2013; Müller-Putz et al., 2005).

Various studies have demonstrated clinical efficacy and support for use of BCI in stroke rehabilitation (Remsik et al., 2016; Soekedar et al., 2015; van Dokkum et al., 2015). To date, most studies have explored the benefits of the BCI rehabilitation approach in conjunction with conventional interventions, therefore significant evidence of superiority of BCI is still difficult to prove. Larger clinical studies are required with BCI-based neurorehabilitation interventions. There is room for potential use of BCI-based interventions on a smaller, more personalized scale as technology improves and allows for increased portability and compatibility. With increased accessibility to these therapies, a more diverse population will be able to benefit from this type of BCI treatment (Remsik et al., 2016).

CONCLUSION

Stroke is one of the leading causes of disability worldwide. Motor impairment due to stroke affects the patient's mobility, ability to perform daily activities and participation in society. All of these factors will impact greatly on the patient's quality of life. Rehabilitation of the arm following stroke is a complex intervention that integrates different modalities to treat deficits that are often multi-factorial and where different elements need to be considered, such as cognitive impairment, global motor function, patient outlook and adjustment to disability. Currently, approaches in treatments aimed at upper limb recovery are beginning to combine traditional methods with other complementary techniques described above in order to tailor treatment to the needs of the individual stroke patient.

Interdisciplinary collaboration is a key point in the treatment process. The availability of specific and up-to-date scientific evidence is crucial for professionals in order to make adequate evidence-based clinical decisions.

Implementation strategies should be put in practice in order to optimise the transfer of scientific knowledge into clinical practice. Several new techniques offer great promise for the future of stroke rehabilitation, although there are still many questions to be answered such as the clinical effect, the long-term outcomes, the cost-benefit of many of these interventions on functional recovery and the precise mechanisms of action of the various therapeutic strategies.

The methods and means available for the rehabilitation of these patients have varied hugely in recent years, with greater emphasis being placed on technological devices. It is still necessary to perfect and improve the design of these devices and to establish cost-effective treatment protocols in order to make them more accessible to daily clinical practice.

Scientific evidence related to stroke recovery will continue to grow in the coming years thanks to advances in neuroscience and neuro-imaging tests, as well as the increasing number of studies which are being undertaken on emerging new therapies that will contribute more data to neurorehabilitation and result in an improvement in the quality of life of these patients. In pursuit of this goal, an interdisciplinary effort combining various professionals from the fields of medicine, neuroscience and engineering is needed in order to achieve further progress.

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Chapter 106

COGNITIVE IMPAIRMENT AND DEMENTIA FOLLOWING ISCHEMIC STROKE AND CARDIAC ARREST IN HUMANS

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ABSTRACT

As the population is aging worldwide, the burden of cardiovascular diseases is constantly increasing. Ischemic heart disease and ischemic stroke are among the major causes of premature death and significant morbidity, resulting in already extremely high and increasing health care and social costs. One of the most challenging issues associated with the ischemic insult to the brain is the long-term cognitive impairment, observed in both stroke and cardiac arrest survivors. Recently, it has been shown that an ischemic brain event can be associated with the manifestation of a type of Alzheimer's disease neuropathology, possibly facilitating the development of dementia due to Alzheimer's disease. It also seems feasible to assess genetic predisposition and factors associated with an increased risk of developing Alzheimer's disease in the stroke survivors' population, similarly to cardiac arrest victims.

Future research efforts should focus on searching for pharmacologic and non-pharmacologic interventions to improve cognition, mental health, and quality of life of stroke and cardiac arrest survivors.

Keywords: Cardiac arrest, ischemic stroke, cognitive decline, dementia, Alzheimer's disease

INTRODUCTION

Both ischemic heart disease and stroke are increasingly prevalent in aging societies. Since they belong to the leading causes of death and disability worldwide, it is not surprising

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that their economic burden is tremendous (Mozaffarian et al. 2016). There has been a significant improvement in the level of care delivered to the patients in the acute phase of the beforementioned diseases, resulting in significantly better survival rates. Unfortunately, the high prevalence of significant cognitive impairment observed in cardiac arrest and stroke survivors remains a challenge for both scientific and clinical communities. Numerous studies were published on the pathophysiology of the neuronal injury resulting from hypoxic-ischemic insult, and a lot of therapeutic approaches were investigated in both experimental and clinical settings (Thiel et al. 2014, Cronberg 2017). Unfortunately, in most cases, the primary goal was to increase the survival rates. Therefore, it should not be surprising that the results were either negative or inconclusive. It seems clear that future research should be focused on prevention of neurological damage and effective therapeutic approaches, which could improve neurological outcome after the brain insult. The primary objective of this chapter is to discuss the epidemiology of ischemic stroke and sudden cardiac arrest, as well as related mortality and morbidity, with a particular focus on the prevalence of dementia following the acute hypoxic-ischemic insult to the brain.

CARDIAC ARREST

Cardiovascular disorders are the most common cause of death worldwide (Mozaffarian et al. 2016). They contribute to approximately 75-80% of all cardiac arrests occurring in the pre-hospital setting, with the estimated global incidence of 62 per 100,000 person/years (Berdowski et al. 2010). In the United States, sudden cardiac arrest affects approximately 350,000 people each year, and mortality is attributed to 300,000 beings. Therefore, the above numbers give a meager survival rate of an estimated 8% (Nichol et al. 2008). The European survival rates after out-of-hospital sudden cardiac arrest are strikingly similar and remain as low as 10% on average, with the number of 275 000 people affected every year (Wissenberg et al. 2013). The high prevalence of premature deaths associated with the sudden cardiac arrest of cardiovascular origin has been the major contributor to launching new resuscitation guidelines. The broad implementation of the "chain of survival" concept, which includes bystander cardiopulmonary resuscitation, widespread access to automatic external defibrillators, and early coronary angiography with subsequent angioplasty, resulted in improved survival rates. National registries from Europe and USA show a 10% decline in mortality in the out-of-hospital cardiac arrest victims in the past two decades (Daya et al. 2015).

Nevertheless, despite many advances in the level of care delivered to the patients in both prehospital care and in-hospital management, the sudden cardiac arrest is still associated with high morbidity and mortality. There is a growing body of evidence that cognitive and functional impairment after cardiac arrest remains one of the significant areas of concern for physicians (Perez et al. 2016). The primary objective of the studies published in the past was to improve survival. In the real clinical setting, it seems more feasible to search for methods capable of enhancing the neurologic outcome, as the severity of hypoxic-ischemic brain injury is the primary determinant of survival. Therefore, it should not be surprising that research focus has shifted beyond prognostication in the acute period following successful cardiopulmonary resuscitation to the identification of mechanisms for long-term brain injury.

Another promising area of research is the implementation of therapies with a potential to reduce neuronal damage following hypoxic-ischemic brain injury.

The brain is extremely vulnerable to lack of constant supply of oxygen and glucose, due to its high metabolic rate and minimal energy supplies. When oxygen and glucose are not supplied in sufficient amounts to provide adequate ATP synthesis in the neurons, the patient loses consciousness, and the disappearance of electrical activity in the cerebral cortex on the electroencephalogram is observed within a few seconds. If blood flow is not promptly restored, exaggerating energy depletion will lead to anoxic depolarization with loss of membrane potentials, the release of excitatory glutamate, loss of ion gradients, and the influx of water and calcium ions, triggering further intracellular pathological processes. The beforementioned mechanisms explain how the sudden stop of cerebral blood flow, resulting from loss of pulse during a sudden cardiac arrest, leads to brain damage due to global cerebral ischemia (Busl and Greer 2010). It should be noted that the neurons in different parts of the brain vary regarding their vulnerability to ischemic-anoxic injury. The most profound damage is exerted to the hippocampus, cerebellum, and neocortex, while the brain stem neurons may remain intact for a significantly more extended period. The differential vulnerability to sudden cessation of blood flow explains why brain stem functions usually recover hours to days earlier than higher cortical functions. Therefore, the survivors of cardiac arrest in a minimally conscious or vegetative state may be successfully weaned from the mechanical ventilation and do not require prolonged circulatory support.

In patients successfully resuscitated from cardiac arrest, there is a broad spectrum of symptoms associated with neurological injury due to global brain ischemia and reperfusion. Since time from the collapse to return of spontaneous circulation is of the essence, the immediate assessment of neurological function may range from brain death to normal cognition, depending on factors such as the bystander cardiopulmonary resuscitation, the availability of automatic external defibrillator, and the identification of the potential reversible cause of the cardiac arrest (Lim et al. 2014). However, in most cases, patients who have been successfully resuscitated will be initially unconscious, apneic and display symptoms of hemodynamic instability, thus they will have to be admitted to the intensive care unit (ICU). In the initial period following the ICU admission, the leading cause of death is the circulatory/multiorgan failure, whereas during the later stages of hospital stay the poor prognosis is mainly determined by the magnitude of brain injury (Dragancea et al. 2013). Therefore, it should not be surprising that more than 50% of patients will not survive to hospital discharge. It has been recently advocated that to improve the patients' prognosis during the ICU stay the focus should shift from general circulatory, respiratory and metabolic optimization to evaluation of the extent and development of brain injury (Cronberg 2017).

The remaining survivors are not likely to be deemed fully recovered immediately after hospital discharge. They usually exhibit cognitive decline or impairment that reaches from mild to severe, including memory loss, decreases in psychomotor function, executive function, and visuospatial function. The beforementioned disturbances influence the vast majority of the affected population, with recent estimates as high as 88% (Andersson et al. 2015). It is beyond doubt that memory loss is the most commonly reported cognitive deficit among patients who regained consciousness following cardiac arrest. It is noteworthy that delayed recall and recognition memory are usually affected the most, while isolated memory loss is uncommon. It may be concluded that the usual pattern of impairment following cardiac arrest includes memory, subtle motor, as well as executive function deficits (Lim et al. 2004).

The assessment of neurological outcomes at hospital discharge is essential regarding immediate prognostication, but it seems increasingly important to improve the understanding of the full spectrum of cognitive and functional deficits observed in cardiac arrest survivors. According to recent studies, patients should undergo a careful assessment of deficits in long-term and short-term memory, cognitive impairment and possible progression to dementia, executive dysfunction, depression, decreased quality of life, and social participation (Perez et al. 2016). The prognosis for regaining cognitive functions to the pre-insult level is usually uncertain. The speed of cognitive rehabilitation usually reaches a plateau at the 3-month mark following resuscitation, yet lengthy rehabilitation has been shown to improve overall performance in a broad spectrum of activities and should be sought (Fertl et al. 2000). Many researchers have addressed the issue of prolonged disability due to cognitive deficits in cardiac arrest survivors at different time points. It seems evident that in the most cases patients are unable to resume their normal daily activities within the first year after the insult and the deficiencies specified earlier may be detectable for up to 8 years following the cardiac arrest (Lundgren-Nilsson et al. 2005, Mateen et al. 2011). A recent study from Sweden with an extremely long-term follow up of a mean of 17 years following a cardiac arrest showed a tendency towards lower scores on the cognitive testing and lower self-reported quality of life (Andersson et al. 2015). However, the authors found no evidence for an increased prevalence of depression, post-traumatic stress disorder or anxiety in the studied population. On the over hand, they concluded that cardiac arrest survivors might exhibit permanent cognitive impairments, including a progression to an Alzheimer's disease-type dementia (Andersson et al. 2015). The risk of dementia may be higher in the beforementioned population, because of the hypoxic event sustained during the collapse. A recent hypothesis links hypoxia to Alzheimer's disease-type molecular cortical abnormalities, perhaps facilitating the development of a dementia phenotype. It was demonstrated that transient hypoxia is associated with hyperphosphorylation of cytoskeletal proteins and abnormalities in neuronal mitochondrial function, which can lead to Alzheimer's disease-type lesions, as well as may elevate the level of amyloid β , a primary substrate in Alzheimer's disease pathology (Chen et al. 2003). Another possible pathway in Alzheimer's disease pathogenesis after ischemia is the dysregulation in the gene expression of amyloid protein precursor, β -secretase, presenilin 1 and 2 and tau protein, following global brain ischemia in the experimental model of cardiac arrest (Kocki et al. 2015, Pluta et al. 2016a, Pluta et al. 2016b, Pluta et al. 2018). Therefore, the cognitive decline following cardiac arrest is characterized by greater memory impairment than executive dysfunction, making it similar to prodromal Alzheimer's disease.

ISCHEMIC STROKE

A recent report on the global burden of stroke concerning incidence, prevalence, mortality, disability-adjusted life-years lost, and mortality-to-incidence ratios showed a growing burden of stroke globally and in low-income and middle-income countries in particular (Feigin et al. 2014). Another disturbing information is that although the mean age of patients with ischemic stroke is increasing in all countries, the proportion of patients younger than 65 years remains substantial, and more than 83 000 children and youths aged 20 years and younger are affected by stroke annually. The authors of the beforementioned report

have unquestionably demonstrated that despite encouraging data showing a significant decrease in mortality rates and mortality-to-incidence ratios, the absolute number of patients affected every year, stroke survivors, related deaths, and disability-adjusted life-years lost are vast and increasing over time. They also concluded that if these trends continue, by the year 2030 there will be almost 12 million stroke deaths, 70 million stroke survivors, and more than 200 million disability-adjusted life-years lost annually around the globe (Feigin et al. 2014). The data on increasing prevalence of stroke among patients aged 18-50 years old was recently confirmed by other authors, who estimated the incidence of stroke among young adults to be more than two million cases per year (Béjot et al. 2016). These cases are of extreme interest to the public health care and social systems, due to a considerable socioeconomic impact because of high health care costs and loss of labor productivity (Ekker et al. 2018). It should not be surprising that stroke-induced cognitive decline, which often progresses into dementia, is responsible for the substantial part of the costs associated with the healthcare and social services. The European data showed that the total annual cost of dementia, including post-stroke dementia, was about 105 billion euros in 2010 (Olesen et al. 2012). These costs will continue to rise in the future, because of the increasing prevalence of stroke and dementia in the aging population. Stroke and dementia often co-exist, and prevalence data show that at the time of the first stroke 10% of patients are already diagnosed with dementia, another 10% will develop dementia after the first stroke, and finally, 1/3 of patients will develop dementia following the stroke recurrence (Pendlebury and Rothwell 2009). Cognitive impairment following both ischemic and hemorrhagic stroke usually involves more than one cognitive domain. It is estimated that up to 65% of stroke survivors demonstrate multi-domain cognitive impairment that mainly contributes to increased caregiver burden and dependence in daily life activities and it may also significantly interfere with the potential benefits of physical rehabilitation and subsequent functional recovery (Donovan et al. 2008).

The data on the prevalence of post-stroke dementia are often elusive because they depend on the setting, the population, the exclusion criteria, the criteria used for the diagnosis of cognitive impairment, and the time interval since stroke (Henon et al. 2006). The lowest incidence (7%) is reported in patients after the first insult, while the highest one (41%) is associated with recurrent stroke and pre-stroke dementia. Interestingly, after the first episode of post-stroke dementia, the cumulative incidence increases linearly at a rate of 3% and 1.7% per year in hospital-based and population-based studies, respectively (Pendlebury and Rothwell 2009). In one of the few long-term studies on the incidence of post-stroke dementia, the cumulative incidence at year 25 was estimated at 48% (Kokmen et al. 1996).

The major problem in exploring the phenomenon of post-stroke dementia is that the borders between degenerative and vascular dementias often overlap, resulting in a mixture of pathologies in 20% of patients with cognitive impairment (Koistinaho and Koistinaho 2005). The pathophysiology of the cognitive decline in this challenging patient population may be a result of vascular, degenerative and inflammatory processes that may be present alone or in different combinations (Koistinaho and Koistinaho 2005). It was also reported that cerebral hypoperfusion, due to unilateral carotid stenosis, results in increased amyloid accumulation, which makes a clear association between stroke and Alzheimer's disease (Huang et al. 2012). The results of experimental studies have shown that the presence of amyloid augments the neuroinflammatory response after stroke (Whitehead et al. 2007). The abovementioned findings led to the formulation of a hypothesis for the development of post-stroke cognitive decline, which states that stroke triggers additional pathophysiological processes, capable of

initiating a secondary degenerative process, which subsequently may interact with pre-existing Alzheimer's disease pathology and thus accelerate neurodegeneration (Thiel et al. 2014). Therefore, it seems feasible to assess genetic predisposition and factors associated with an increased risk of developing Alzheimer's disease in the stroke survivors' population, similarly to cardiac arrest victims. It has to be kept in mind that in contrast to cognitive impairment caused by Alzheimer's disease, vascular cognitive impairment is associated with vascular factors and does not mainly affect memory but many different cognitive domains. However, some domains such as executive functions or speed of mental processing are more likely to be affected (Brainin et al. 2015).

CONCLUSION

The prevalence of ischemic heart disease and ischemic stroke and their burden for health care and social systems worldwide is increasing due to many different factors. This phenomenon is partly because of hypoxic-ischemic brain injury, resulting from the abovementioned diseases, almost inevitably leads to global neurological dysfunction and persistent cognitive decline. Future research efforts should focus on searching for pharmacologic and non-pharmacologic interventions to improve cognition, mental health, and quality of life of stroke and cardiac arrest survivors.

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Chapter 107

POST-ISCHEMIC DEMENTIA WITH ALZHEIMER'S DISEASE PHENOTYPE FOLLOWING ISCHEMIC STROKE IN HUMANS AND EXPERIMENTAL ISCHEMIC BRAIN INJURY

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ABSTRACT

In this chapter, we compared motor and non-motor symptoms between experimental brain ischemia and ischemic stroke in patients, and noted that ischemia-reperfusion injuries influenced cognition and dementia development. Ischemic stroke in humans is a leading cause of disability. Dementia after ischemic stroke affects up to one third of stroke survivors. Investigations and therapeutic actions have historically focused mainly on physical disabilities, when cognitive deterioration, an important symptom for post-stroke survivors, has been quite neglected. Even a minor stroke affects daily functioning, executive functions, and cognition, consequently affecting participation, the quality of life, and return to work. Stroke survivors are at an increased risk of developing cognitive impairment. Obviously, the acute tissue damage may affect cognition. Nevertheless, despite prospective data being available, the results are conflicting and the direct

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cognitive effect of a stroke event beyond the cognitive decline associated with age and vascular risk factors remains poorly understood. Physical impairments following ischemic stroke tend to improve to a greater or lesser degree. However, for reasons which remain unknown, cognitive impairments progressively worsen. One year after experimental brain ischemia rats showed: hearing dysfunction, hyperactivity, problems with adaptation to a new environment, reduced anxiety, learning and memory impairment in a battery of hippocampal dependent tests. Post-stroke dementia in humans is associated with factors indicating a reduced cognitive reserve (low education, white matter lesions, and brain atrophy) and is also strongly associated with stroke factors (damage size, multiple lesions and stroke recurrences) indicating the likely impact of optimal acute stroke care and secondary prevention in reducing the burden of dementia.

Keywords: dementia, stroke, transient ischemic attack, brain ischemia, cognition, behavior, personality, brain atrophy, human, experimental study

INTRODUCTION

Dementia is a common term for symptoms presented by patients with different kinds of cognitive disability. These symptoms include impaired mental activities in such areas as: learning, memory, attention, judgment, concentration, thinking and language. They are often accompanied by changes in personality and behavior. It is a debilitating neurodegenerative syndrome which causes a gradual and irreversible decline in cognitive, physical and social function (Landeiro et al., 2018). Dementia is a condition with little prospect of a cure or preventing its progression (Landeiro et al., 2018) and is caused by more than 60 different disorders. It appears mostly in people over 65 years of age, and an increase in figures and costs is associated with greater longevity as a result of reduction in the number of people dying prematurely. In 2014, it was estimated for circa 7% of people aged 65 and above worldwide (Landeiro et al., 2018). There are different types of dementia and some patients may have a combination of types. Alzheimer's disease type dementia is the most common and accounts for more than 60-80% of diagnosed dementias (Landeiro et al., 2018). Next common form of dementia is vascular dementia, usually as a succession, connected with different ischemic brain lesions. Dementia also has an effect on social and economic costs. It is forecast that the dementia will advance exponentially in the coming decades, when the older part of the population will continue to grow in age. The challenge facing Europe and the world lies in developing an interdisciplinary combination of clinical, medical, economic, governmental, social and personal approaches to those with dementia and their families. Dementia does harm not only to patients but to caretakers, families, communities and national healthcare systems.

In 2010, circa 17 million cases of ischemic stroke have been reported worldwide and the number of patients living after an ischemic stroke accounted for 33 million (Brainin et al., 2015, Bejot et al., 2016). The annual cost of dementia, turning to post-stroke dementia, was calculated to be 105 billion dollars in Europe in the above year (Brainin et al., 2015). Today, dementia affects circa 50 million people worldwide, with this number expected to increase to 75 million by 2030 and to 132 million by 2050 (Frankish, Horton 2017, Livingston et al., 2017), which is driven to a large extent by population ageing. Dementia as a syndrome of brain damage causes not only disability and dependency for individuals affected by the

disease, but it can also have a strong detrimental effect on handlers, especially on family caretakers, who are at a high risk of developing anxiety disorders and depression (Frankish, Horton 2017). Currently the cost of caring for patients with dementia is more than 800 billion dollars per year worldwide (Livingston et al., 2017), increasing to 2 trillion dollars by 2030 (Frankish, Horton 2017). Its high prevalence, progression and enormous requirements for care and social support lead to a public health crisis. Dementia is a huge worldwide challenge for healthcare system and social care in the 21st century.

Society is aging, the population lives longer and dementia is growing rapidly. The incidence of dementia is difficult to determine finally because many cases remain undiagnosed. Dementia is not caused by aging, nor is it an integral part of the aging processes. It is more likely a combination of environmental, genetic and age-associated processes. Despite the huge resources currently directed at biomedical research of neurodegenerative diseases such as Alzheimer's disease and brain ischemia, no etiology and cure is in sight. Only some symptomatic and palliative therapies are available, although the knowledge of neurological processes, especially among the elderly population, is increasing rapidly. Actually, the dominant trend is to shift responsibilities from national support policies to the families which are not prepared for that. Currently, dementia is a growing wave and a neglected problem at the same time. Today our goal is to promote efforts to find communal solutions and interventions that preserve people's humanity and delay institutional care.

Ischemic brain injury has been recognized as a trigger of Alzheimer's disease (Pluta et al., 1994, Pluta 1997, Honig et al., 2003, Pluta 2006, Pluta, Ułamek 2006, Pluta 2007a, Pluta 2007b, Pluta, Ułamek 2008, Pluta et al., 2009, Pluta et al., 2010a, Pluta et al., 2010b, Pluta et al., 2013a, Pluta et al., 2013b, Kocki et al., 2015, Pluta et al., 2016a, Pluta et al., 2016b, Ułamek-Kozioł et al., 2016a, Ułamek-Kozioł et al., 2016b, Pluta et al., 2017, Salminen et al., 2017, Ułamek-Kozioł et al., 2017, Pluta et al., 2018). Patients with Alzheimer's disease, who have ischemic lesions, were found to have more intense dementia (Snowdon et al., 1997). Shared susceptibility to Alzheimer's disease and ischemic brain lesions might explain the frequent association of both disorders. In this chapter, we used data from different laboratories and clinics to compare the relationship between dementia after ischemic brain injury with dementia associated with Alzheimer's disease. If the history of stroke proceeds the development of dementia such a relationship cannot be excluded. It was noted that the history of ischemic stroke was associated with the subsequent development of Alzheimer's disease, primarily in the presence of other risk factors related to both cardiovascular and ischemic brain injuries. On the other hand, it is possible that Alzheimer's disease might increase the likelihood of ischemic stroke. The presence of neuropathological changes in Alzheimer's disease brain could predispose some patients to ischemic stroke, perhaps owing to amyloid angiopathy, brain tissue changes, or the secondary consequences of the β -amyloid peptide neurotoxicity. It needs to be established whether ischemic stroke is directly involved in the neuropathogenesis of Alzheimer's disease or acts indirectly as a contributor to the manifested symptoms of Alzheimer's disease. Nonetheless, prevention and/or treatment of ischemic stroke may have important implications for Alzheimer's disease risk and deserve further investigation. For example, pre-existing brain damage by ischemic stroke might additionally increase the likelihood of Alzheimer's disease by increasing the extent of injury from the genomic and proteomic cascade of Alzheimer's disease.

DEMENTIA FOLLOWING ISCHEMIC STROKE IN HUMANS

During three months observations of patients after ischemic stroke it was noted that 57.2% of survivors had a mild disability, 9.4% - moderate disability and 18.6% - severe disability (Surawan et al., 2017). As presented above, ischemic brain injuries in humans are the most common progressive causes of disability worldwide and eventually have a negative impact on the patients, caregivers and society as a whole (Flynn et al., 2008). Brain ischemia patients suffer from progressive neurological deficits that significantly limit their ability to return completely to society (Surawan et al., 2017). An insidious consistency of post-ischemic brain alterations is a slow and progressive development of post-ischemic dementia (Desmond et al., 2002, Jellinger 2007, Pinkston et al., 2009, Zhang et al., 2010, Gemmell et al., 2012, Brainin et al., 2015, Mok et al., 2016, Portegies et al., 2016, Kim, Lee 2018) which is associated with severe neurological disability (Surawan et al., 2017). Dementia is the worst consequence for patients after ischemic brain injury and it is responsible for approximately 20% of all diagnosed dementias (Fillit, Hill 2002). Worldwide, dementia, after ischemic stroke, varies from 5% to 50% depending on the diagnostic criteria, population demography and geographic location (Leys et al., 2002, Zhang et al., 2010, Surawan et al., 2017). In fact, now it is clear that dementia following brain ischemia-reperfusion incidence has many risk factors in common with late development of sporadic Alzheimer's disease. There is a high probability that brain ischemia lesions may precede the onset of Alzheimer's disease type dementia and trigger all the consequences associated with the development of dementia. Dementia following ischemia associated with progressive delayed secondary alterations occurs in the people suffering from transient ischemic attack, lacunar, focal and salient ischemic brain episodes in a progressive manner (Bornstein et al., 1996, Jellinger 2007, Pinkston et al., 2009, Gemmell et al., 2012, Mok et al., 2016, Bivard et al., 2018). The chronic post-ischemic injuries have received far less attention in experimental and clinical dementia investigations. Epidemiological studies have presented that the prevalence of dementia in survivors after brain ischemia is about 9 times higher than in controls without stroke after 3 months (Tatemichi et al., 1992, Pohjasvaara et al., 1998, Madureira et al., 2001, Surawan et al., 2017), and after a lacunar infarct 4–12-fold higher than in healthy people after 4 years (Loeb et al., 1992). In long term-based observations of survivors after ischemic stroke it was demonstrated that the incidence of dementia amounted to 7% after 1 year (Tatemichi et al., 1990). It was reported that 32% of survivors, who were at the beginning free of dementia, developed it over 5 years after the first ischemic incidence (Bornstein et al., 1996). Another study has shown that the combined proportion of survivors with dementia constituted 21% during 3 years of observation (Henon et al., 2001). The examined survivors after brain ischemia develop dementia in around 22% by the end of 4 years in the follow-up time (Altieri et al., 2004). In the studies of patients at different times after ischemic stroke it was documented that the incidence rate of dementia achieved 7% in 1 year, 10% in 3 years, 15% in 5 years and 23% in 10 years of observation (Kokmen et al., 1996). In two different studies of survivors after a lacunar brain ischemia episode, it was found that 5-10% of cases had dementia after 1-3 years of observation (Samuelsson et al., 1996), and in the other report that 23% of survivors developed dementia during 4 years of survival (Loeb et al., 1992). Still, the incidence of dementia following recurrent ischemic stroke was 33.33% (Surawan et al., 2017). Global ischemia in childhood frequently leads to poor neurologic outcomes, including

memory and learning deficits (Dietz et al., 2018). In the novel model of childhood transient global brain ischemia memory is impaired during seven days after ischemia and completely recovers through 30 days (Dietz et al., 2018).

DEMENTIA FOLLOWING EXPERIMENTAL ISCHEMIC BRAIN INJURY

In addition to neuronal injuries caused by ischemia and recirculation, behavioral alterations have been observed, too (Block 1999, Barra de la Tremblaye, Plamondon 2011, Kiryk et al., 2011, Li et al., 2011, Pluta et al., 2011, Cohan et al., 2015). Injury after ischemia and recirculation does not result in long-lasting neurological deficits in experimental animals (Block 1999). Spontaneous restoration of sensorimotor activities has been observed in the period following ischemia (Popa-Wagner 2007, Yang, Simpkins 2007, Kiryk et al., 2011). After ischemia-reperfusion brain alterations in animals, locomotor hyperactivity has been noted (Kuroiwa et al., 1991, Karasawa et al., 1994) such as in Alzheimer's disease patients. The hyperactivity was related to the death of pyramidal neurons in hippocampus (Kuroiwa et al., 1991). A longer time of ischemia and, consequently, a longer duration time of locomotor hyperactivity is directly correlated with a raised number of neuron loss in hippocampus and neuroinflammation (Block 1999, Langdon et al., 2008, Pluta et al., 2010a, Sekeljic et al., 2012). After ischemia-reperfusion brain injury, impaired habits, as manifested through an increase in examination time, were shown (Milesen, Schwartz 1991, Colbourne, Corbett 1995). Brain ischemia causes reference and working memory deficiency (Davis et al., 1986, Kiyota et al., 1991, Kiryk et al., 2011). Ischemia-reperfusion brain injury in experimental animals leads slowly to spatial memory deficits over the recirculation period (Block, Schwarz 1998, Karhunen et al., 2003, Kiryk et al., 2011). Progression of cognitive deficits has been observed every time during recirculation phase (Roof et al., 2001, Karhunen et al., 2003, Kiryk et al., 2011). In addition to that, observations of repetitive transient brain ischemia injury in animals have shown durable locomotor hyperactivity, persistent cognitive deficits and decreased anxiety (Ishibashi et al., 2006). The behavioral abnormalities, mentioned above, were related to an enormous brain atrophy (Hossmann et al., 1987, Pluta 2000, Pluta 2002, Pluta et al., 2009, Jabłoński et al., 2011, Pluta et al., 2012a, Pluta et al., 2012b), neuron loss in the CA1 area of hippocampus, caudate nucleus, brain cortex (Ishibashi et al., 2006, Pluta et al., 2009, Pluta et al., 2010a, Pluta et al., 2012b), amygdala and perirhinal cortex (Barra de la Tremblaye, Plamondon 2011). Alertness and sensorimotor capacities are affected within 1–2 days whereas the deficits in learning and memory appear to be slowly irreversibly progressing and lasting for good (Langdon et al., 2008, Kiryk et al., 2011).

CONCLUSION

Removal and prevention of the dementia and neurological consequences after brain ischemia is an issue that scientists and neurologists spend only little time on. In some survivors, a spontaneous neurological restoration is shown within weeks and/or months after an ischemic episode. Notwithstanding, in general, a spontaneous restoration and/or recovery is partial. Furthermore, brain ischemia episode frequently results in a complete functional

devastation of its victims and, as a consequence, is the leading cause of permanent disability of survivors who require permanent institutional care. The loss of quality of life over the years and financial resources spent on healthcare are staggering. The circumstance is even deteriorated by the fact that no causal treatment is available for the cases during and after acute ischemic brain injury. The social burden after brain ischemia increases dramatically. Thus, the knowledge of the underlying progressive neuropathological mechanisms is urgently required. This chapter tends to collect together the pathological alterations during recirculation period and reveals convincing molecular mechanisms. To summarize, the basis of evidence from both experimental and clinical studies revealed that the slow progressive impairment of cognitive functions could not be explained only by the direct influence of the ischemic brain injury, but rather by a progressive effect of the additive factors after ischemic injury, e.g., Alzheimer's disease related factors, and finally, aging (Pluta et al., 1994, Wiśniewski et al., 1995, Pluta 1997, Honig et al., 2003, Pluta 2006, Pluta, Ułamek 2006, Jellinger 2007, Pluta 2007a, Pluta 2007b, Popa-Wager 2007, Langdon et al., 2008, Pluta, Ułamek 2008, Pluta et al., 2010b, Pluta et al., 2011, Gemmell et al., 2012, Pluta et al., 2012a, Pluta et al., 2012b, Pluta et al., 2013a, Pluta et al., 2013b, Kocki et al., 2015, Pluta et al., 2016a, Pluta et al., 2016b, Portegies et al., 2016, Ułamek-Kozioł et al., 2016a, Ułamek-Kozioł et al., 2016b, Ułamek-Kozioł et al., 2016c, Pluta et al., 2017, Salminen et al., 2017, Ułamek-Kozioł et al., 2017, Pluta et al., 2018). The above suggestions have been confirmed by ischemic overexpression of amyloid protein precursor and its amyloidogenic processing secretases' genes, which may be strongly involved in the deterioration of cognitive activities in the recirculation period (Shi et al., 1998, Shi et al., 2000, Pluta et al., 2013a, Pluta et al., 2013b, Kocki et al., 2015, Pluta et al., 2016a, Pluta et al., 2016b). The production of β -amyloid peptide after brain ischemia increases and influences negatively the memory (Yan et al., 2007). Additionally, a pathological expression of tau protein gene after ischemia (Pluta et al., 2018) and deposition of α -synuclein might disturb the synaptic function, resulting in cognitive deficits (Hashimoto, Masliah 1999). The functional changes precede the neuronal cell final degeneration within the sectors of selective vulnerability to ischemic episodes. Furthermore, regions of brain, that do not have ischemic neuronal cell injury, demonstrate functional abnormalities. The above abnormalities seem to be mostly caused by synaptic insufficiency in communication of neurons between ischemic sectors with damaged or dead neurons.

During the last 25 years, a growing body of evidence has implied a close relationship between brain ischemia episodes and Alzheimer's disease. Different investigations, including clinical epidemiological studies, have paid attention to a relationship between neurovascular risk factors and Alzheimer's disease development. The link between Alzheimer's disease and cerebrovascular diseases has been supported by the following facts: (1) Alzheimer's disease may increase the likelihood of suffering from ischemic stroke and vice versa (Pluta 2004a, Pluta 2004b), (2) the deposition of the β -amyloid peptide in the brain tissue of Alzheimer's disease patients toxically affects neurons (Hardy, Selkoe 2002) and vascular system (Paris et al. 2004a, Paris et al., 2004b), and what is even more, (3) Alzheimer's disease and atherosclerosis share environmental and genetic risk factors like apolipoprotein E e4 polymorphism, hypertension, hypercholesterolemia, metabolic syndrome and obesity (Casserly, Topol 2004).

Data from this chapter demonstrate a relationship between the history of ischemic stroke and Alzheimer's disease development. Thus, there was an increased risk of Alzheimer's

disease development with the severity of dementia symptoms (Snowdon et al., 1997) in persons with the history of ischemic stroke. The risk was the highest for those patients with ischemic stroke who also had established vascular risk factors, such as heart disease, high blood pressure or type 2 diabetes mellitus. The relationship of Alzheimer's disease risk factors with stroke remained the same. The worldwide problem and enormous costs involved make it clear that there is an urgent need for advances in the prevention and/or treatment of brain ischemia-reperfusion injury and its irreversible consequences like post-ischemic dementia. The growing problem of dementia which occurs following ischemic stroke forces an increase of funding resources for clinical and basic research. Recently, no established methods of prevention and no cure have been available once the dementia becomes manifested. Significant cortical, hippocampal and medial temporal lobe atrophy with white matter changes are observed in the development of cognitive decline after ischemic stroke (Hossmann et al., 1987, Pluta 2000, Pluta et al., 2008, Pluta et al., 2009, Bivard et al., 2018). The dynamics of the above ischemic changes and their further progress are being strongly studied at present. Future studies are required to clarify the interaction between degenerative, vascular, and systemic processes in the development of stroke-associated dementia. Genetics related to Alzheimer's disease and factors associated with an increased risk of developing Alzheimer's disease also need to be investigated.

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Chapter 108

STROKE AND ALZHEIMER'S DISEASE: COMMON MECHANISMS AND THERAPEUTIC APPROACHES

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ABSTRACT

Stroke and Alzheimer's disease (AD) have been placed in different areas of neurology. They are considered to be examples of different forms of brain dysfunction - stroke, as an acute cerebrovascular injury, and dementia as a progressive, chronic neurodegenerative disease. However, there are overlaps in pathophysiological mechanisms between these brain conditions. Moreover, stroke increases the likelihood of developing AD, by initiating degenerative dementia. There is evidence that stroke risk factors such as age, hereditary factors, hypertension, diabetes mellitus, lipid disorders, obesity, atrial fibrillation, ischemic heart disease, tobacco smoking, and physical inactivity, are independently associated with an increased risk of AD, up to fivefold in the elderly.

Stroke leads to increased extracellular levels of glutamate causing excitotoxicity, peri-infarct depolarisations, inflammation, mitochondrial dysfunction, oxidative/nitrosative stress, and apoptosis. On the other hand, AD is characterized by progressive cognitive deficits, accumulation of amyloid- β and intracellular neurofibrillary tangles, and neuronal death. Increasing evidence suggest that amyloid-independent factors such as stroke-related, also contribute to AD pathogenesis. There are many similarities in different molecular mechanisms of neuronal cell death observed in stroke and AD.

The possible therapeutic targets should be in connection with the common underlying pathophysiological mechanisms in stroke and AD, such as excitotoxicity, oxidative stress, neuroinflammation, and apoptosis. Moreover, new and creative approaches are needed for the stimulation of endogenous neurogenesis and synaptogenesis, as well as for exogenous neuroreplacement.

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Keywords: stroke, Alzheimer's disease, brain injury, neurodegenerative disease, dementia, cognitive impairment, pathophysiological mechanisms, excitotoxicity, oxidative stress, neuroinflammation, amyloid- β peptide, tau protein, animal models, neuroprotection, therapies, stem cells

INTRODUCTION

Stroke and Alzheimer's disease (AD) have been placed in different areas of neurology. They are considered to be examples of different forms of brain dysfunction - stroke, as an acute cerebrovascular injury, and dementia as a progressive, chronic neurodegenerative disease. However, there are overlaps in pathophysiological mechanisms between these brain conditions. Moreover, stroke increases the likelihood of developing AD, by initiating degenerative dementia (Pluta et al., 2009). Dementia in elderly stroke survivors is associated with medial temporal lobe atrophy (Saito et al., 2014). Hippocampal neuronal atrophy is apparent for dementia in both cerebrovascular and neurodegenerative diseases (Gemmell et al., 2012; Kocki et al., 2015). Given the high prevalence of AD, identifying prognostic markers in stroke patients would be a substantial step forward (Cumming and Brodtmann, 2011).

Stroke is the leading cause of mortality and morbidity worldwide. It is also a leading cause of disability, with a high prevalence of new or recurrent stroke events (Lakhan et al., 2009). Yet, the only drug of the US Food and Drug Administration approved for pharmacological treatment of stroke is tissue plasminogen activator, which due to the limited therapeutic post-stroke time window (≤ 4.5 h) is only administered to 2–5% of patients. Administration beyond this period has accompanying complications, such as the hemorrhagic transformation (Brainin et al., 2007; Sehba et al., 2013). So, it is necessary to continue searching for novel therapeutics.

Stroke is, also, an important cause of cognitive deficits. The term cerebrovascular dementia is used to describe dementia attributed to stroke, or deep white matter lesions detected with imaging methods (Sahathevan et al., 2012). The relationship between AD and cerebrovascular dementia is unclear, although significant overlaps exist. There is evidence that the stroke risk factors such as age, hereditary factors, hypertension, diabetes mellitus, lipid disorders, obesity, atrial fibrillation, ischemic heart disease, tobacco smoking, and physical inactivity, are independently associated with an increased risk of AD, up to fivefold in the elderly (Kalogeris et al., 2012; Ulamek-Kozioł, et al., 2016a). Evidence suggests that these risk factors have a cumulative effect on AD development, but not on cerebrovascular dementia (Sahathevan et al., 2012; Ulamek-Kozioł, et al., 2016c).

The primary consequence of stroke in patients, animal models, as well as *in vitro* models is a reduction in energy substrates, including glucose and oxygen (Dirnagl et al., 1999; Lipton, 1999; Korenic et al., 2015). In addition, ATP synthesis through glycolysis and oxidative phosphorylation is dysregulated, with a rapid decline in cellular ATP levels. Stroke leads to increased extracellular levels of glutamate causing excitotoxicity, peri-infarct depolarisations, inflammation, mitochondrial dysfunction, oxidative/nitrosative stress, and apoptosis (Kagaya et al., 2016).

AD is a neurodegenerative disease with clinical hallmarks of memory loss, dementia, and cognitive deficits. It is a chronic disease with well-defined pathophysiological mechanisms, mostly affecting medial temporal lobe and associative neocortical structures (Skaper, 2012; Pluta et al., 2016). Neuronal injury can be observed in the basal forebrain, frontal lobe, hippocampus and cerebral cortex of both patients and animal models (De-Paula et al., 2012; Ulamek-Kozioł, et al., 2016b). AD is characterized by progressive cognitive deficits, accumulation of amyloid- β (A β) and intracellular neurofibrillary tangles, and neuronal death. According to the amyloid hypothesis of AD, the overproduction of A β is a consequence of the disruption of processes that regulate the proteolytic cleavage of the amyloid protein precursor (APP). Genetic, age-related and environmental factors contribute to a metabolic shift favoring the amyloidogenic processing of APP (De-Paula et al., 2012; Pluta et al., 2013a). The resulting protein aggregates are causing synaptic dysfunction, characterized by dendritic spine loss and reduced post-synaptic density, leading to disturbance of network connections and cell death (Duncan and Valenzuela, 2017). The cognitive deficit is a consequence of neural network activity caused by dendrite retraction, dendritic spine and synaptic degeneration, and the death of cholinergic neurons in the basal forebrain and the entorhinal cortex (Huang and Mucke, 2012). Additionally, mitochondrial dysfunction and free radical damage are also hallmarks of AD brain (Hayashi et al., 2006).

The AD pathology is diverse and of multifactorial origin (Pluta et al., 2004a; 2009; 2012; 2013b). Thus, tau protein, an intracellular microtubule-associated structural and transport protein, becomes hyperphosphorylated, leading to microtubule collapse and aggregation into neurofibrillary tangles. On the other hand, cleavage of the APP by secretases leads to the formation of amyloid plaques as extracellular aggregates of A β fragments. The plaques and neurofibrillary tangles are related to the accumulation of the A β peptide in brain, and to cytoskeletal changes. A particular feature of AD is the association of activated microglia and resident macrophages with plaques. Present in the early stages of the disease, their number in AD brains declines with disease advancement. Activated microglia produce humoral immune factors – cytokines, which modulate neuroinflammation. Extensive neuronal and synaptic loss represents the end stage of AD (Duncan and Valenzuela, 2017).

It is clear that different cellular mechanisms can lead to AD, yet no definitive therapy exists. Current therapy is limited to the potentiation of cholinergic transmission in the nucleus basalis to compensate presynaptic cholinergic deficiency, and the prevention of N-methyl-D-aspartate (NMDA) receptor activity during cognitive loss. However, present therapies are limited and may provide only symptomatic relief (Chonga et al., 2005). The pharmacological treatment of AD currently relies on inhibition of acetylcholinesterase (Shah and Reichman, 2006). This inhibition increases the concentration of acetylcholine at cholinergic synapses to promote neurotransmission. In addition, ongoing clinical trials aim to reduce synapse degeneration by targeting APP processing mechanisms, A β production and aggregation (Huang and Mucke, 2012). Although these treatments may improve cognitive function by reducing disease progression, regeneration of damaged neurons is still without pharmacological treatment (Smith et al., 2017).

ANIMAL MODELS OF STROKE AND ALZHEIMER'S DISEASE

With an increasingly aging population, high incidence of stroke, and limited therapies, it is important to improve functional recovery of stroke survivors (Livingston-Thomas and Tasker, 2013). Animal models are very important for understanding the mechanisms underlying neuroprotection and rehabilitation. Neuroprotective drugs are effective in experimental stroke studies, but none have proven clinically effective. A number of reasons have been discussed, such as unpredictable models, weaknesses in the design of experimental studies, as well as problems in achieving effective drug concentrations without severe side effects (Schaar et al., 2010).

Since stroke is a very diverse condition there is no ideal experimental model. Criteria to consider include pathophysiological processes, lesion size, and reproducibility (Livingston-Thomas and Tasker, 2013). While animal models remain crucial to basic stroke research, there are limitations in the translation to clinical practice. Stroke models represent a simplified version of very complex human condition. Gender, age, pre-stroke condition, stroke severity and co-morbidities are highly variable in the clinical population, but controlled in experimental studies (Livingston-Thomas and Tasker, 2013). However, rodents still serve as preferred models for understanding the effects of human stroke (Livingston-Thomas and Tasker, 2013).

AD is an increasing burden for patients and society, as more people live long enough to become affected (Bates et al., 2014). However, there is still the lack of an animal model that accurately replicates AD. Furthermore, as age remains the strongest risk factor for AD, transgenic models in which mutant human genes are expressed throughout the life of the animal provide only limited insight into age-related factors in disease development. Guinea pigs (*Cavia porcellus*) are of interest in AD research, because they have a similar A β sequence to humans and thus, with aging may present a useful non-transgenic animal AD model (Bates et al., 2014). Transgenic mice overexpressing human APP have been used to model A β pathology (Dewachter et al., 2000; Maia et al., 2013), as well as the rabbit model (Bitel et al., 2012). In addition, various higher vertebrate models have been in use to study the pathophysiology of AD, but these models have limitations regarding ethical restrictions, high cost, difficult maintenance and lesser reproducibility. On the other hand, various lower chordate animals such as *Danio rerio*, *Drosophila melanogaster*, *Caenorhabditis elegans* and *Ciona intestinalis* have proven to be important *in vivo* models for the determination of drug targets (Sharma et al., 2017).

Despite the genetic and cellular evidence that supports the amyloid hypothesis, it is becoming increasingly clear that AD etiology is complex and that A β alone is unable to account for all aspects of AD. There is increasing evidence to suggest that stroke also contributes to AD pathogenesis (Skaper, 2012).

THE CONNECTION BETWEEN PATHOPHYSIOLOGY MECHANISMS AND THERAPEUTIC APPROACHES IN STROKE AND ALZHEIMER'S DISEASE

As already pointed out, AD and cerebrovascular dementia are the major causes of cognitive deficits worldwide. Considerable evidence indicates that cerebrovascular

dysfunction and stroke are associated with neurodegenerative processes in aged population, preceding cognitive decline and dementia. Although AD is considered as the most common cause of dementia, AD patients commonly also exhibit cerebrovascular dysfunctions (Sahathevan et al., 2012). AD and cerebrovascular disorders, including stroke, are the leading causes of dementia in the elderly (Pluta et al., 2004b). The progression of neuropathological changes in AD patients initiates years to decades, before the diagnosis of dementia. Therefore, a considerable effort has been made to identify factors that contribute to the pathogenesis of AD. Among these factors are cerebrovascular disorders, stroke, and microvascular alterations affecting the progression of dementia. Cerebrovascular dysfunction has emerged as an early event in AD, including changes in all cell types of the neurovascular unit - astrocytes, pericytes, vascular smooth muscle cells and endothelial cells (Pimentel-Coelho and Rivest, 2012; Pluta et al., 2012).

In addition, persistent systemic hypoxia, a direct consequence of cerebrovascular dysfunction, can compromise the brain by increasing the risk of developing AD. Clinical evidence confirms that structural and functional changes characteristic of AD pathology also occur following stroke (Cumming and Brodtmann, 2011). Moreover, cerebrovascular dysfunction promotes tau protein phosphorylation, modulates apolipoprotein E expression, and has more profound effects in aged animals (Villarreal et al., 2014). In addition, it was found in the ischemia-reperfusion long-lived rats (9 and 13 months after 10 min of cardiac arrest), blood-brain barrier leakage in the area of the dorsal hippocampus and brainstem-hindbrain level in basal cerebellum, as well as inflammatory processes backed by microglial activity, even up to 1 year after ischemia-reperfusion (Sekeljic et al., 2012). Studies with transgenic and non-transgenic mouse models show that hypoxia increases the levels of A β peptides in the brain. Thus, stroke can trigger accelerated amyloid deposition, through interference with amyloid clearance pathways (Pluta et al., 2017). Consequently, there are therapeutic approaches targeting the cerebrovascular system for preventing AD (Pimentel-Coelho and Rivest, 2012).

Longitudinal clinical studies suggest the co-morbidity of stroke and AD (Kalaria, 2000). In addition, more than 30% of stroke survivors will develop dementia within two years (Kalaria, 2012). Stroke and neurodegenerative diseases are additive and accelerate dementia. Medial temporal lobe atrophy is a predictor of dementia. It appears as a feature in demented stroke survivors with minimal AD pathology. The atrophy is attributed to smaller cell volumes in the hippocampus and frontal lobe that reflect loss of neuronal connectivity. Therapeutic strategies that restore functional morphology in surviving neurons could prevent further cognitive decline in stroke and AD (Kalaria, 2012).

The concept of mixed dementia has been confirmed in patients with brain condition showing both AD and cerebrovascular disorder, i.e., cerebrovascular dysfunction along with A β -associated neurotoxicity, and tau protein - hyperphosphorylation. In addition, the important role of neurovascular unit dysfunction in neuronal death and cognitive decline has been indicated. In relation to dementia, the role of astrocytes, the essential cell type in maintaining proper blood-brain barrier and brain function, in A β deposition and clearance is still under investigation (Jo et al., 2014). However, it is known that astrocytes can contribute to neuroinflammation in stroke and AD (Pluta et al., 2018). During brain dysfunction, astrogliosis occurs, followed by an upregulation of the expression of proinflammatory cytokines and chemokines, which are associated with the pathogenesis of both, stroke and AD. Cytokines and chemokines play a role in neuroprotection and neurodegeneration as well,

in the brain. Inflammatory molecules can be either potential biomarkers for diagnosis or target molecules for therapeutic intervention. Understanding their roles and their relationship with activated astrocytes is particularly important for attenuating neuroinflammation in stroke and AD (Li et al., 2011).

During AD, A β , S100B and IL-1 β could accelerate A β generation in neurons leading to chronic neuroinflammation. Furthermore, astrocytes could be reactivated by TGF β 1 to generate more A β and to undergo the aggravating astrogliosis. On the other hand, following stroke, microglia and astrocytes are activated *via* Toll-like receptors (TLRs) (Liu et al., 2011; Wu et al., 2018), leading to up-regulation of nuclear factor kappa B (NF- κ B) (Zwagerman et al., 2010). NF- κ B has been well-documented to be a key regulator of both cell survival and inflammatory genes (Harari and Liao, 2010; Shih et al., 2015). NF- κ B induced inflammation has been shown to be triggered *via* TLR-mediated signaling, which ultimately promotes production of proinflammatory cytokines, such as IL-1 β , IL-6 and TNF- α (Shih et al., 2015; Wu et al., 2018). Thus, astrocytes as well are activated *via* different mechanisms in stroke and AD, hence contributing to the neuroinflammation as one of the hallmarks of both brain conditions. Indeed, anti-inflammatory approaches have proven to be beneficial in the prevention and treatment of stroke and AD-like neuropathology *in vivo* (Koistinaho and Koistinaho, 2005). Still development of effective anti-inflammatory drugs is needed for the therapies of stroke and AD (Wu et al., 2018).

As expected, there are many similarities in molecular mechanisms of neuronal cell death observed in stroke and AD. The neuronal transmembrane potential and ion gradient are disturbed at very early stage of both disorders. Stroke and AD share a number of overlapping mechanisms of neuron loss and dysfunction, including those induced by the extensive activation of glutamate NMDA receptors (NMDARs). Their overactivation with their appreciable Ca²⁺ permeability, leads to neurotoxicity in the cortex and hippocampus. NMDARs have therefore been therapeutic targets in both brain conditions (Janac et al., 2008; Selakovic et al., 2010; Radenovic et al., 2011; MacDonald et al., 2013), but they have failed in the treatment of stroke, and there is limited rationale for using them in treating AD (MacDonald et al., 2013). From point of organelle damage, molecular events seen in stroke and AD are comparable. Mitochondrial and endoplasmic reticulum damage in both disorders share common pathological findings, such as activation of pro-apoptotic signals. Moreover, lysosomes play important roles in stroke and AD. The leakage of lysosomal enzymes can influence the fate of neurons under pathological conditions in both disorders (Hayashi et al., 2006).

Another common pathophysiological mechanism is oxidative stress. As a consequence of stroke, but particularly after reperfusion, reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical, and NO are generated. Radicals react with virtually any cellular component (carbohydrates, amino acids, DNA and phospholipids) and cause direct damage. Free radicals trigger a damaging cycle in the mitochondria, with inhibition of electron transport mechanisms, leading to excess superoxide production and also to activation of mitochondrial permeability transition (mPT) (Hayashi et al., 2006). Besides impeding ATP production, through loss of mitochondrial potential (Korenic et al., 2014), mPT leads to mitochondrial swelling and release of pro-apoptotic molecules (Hayashi et al., 2006). Oxidative stress is closely linked to excitotoxicity, energy loss and ionic imbalances, and all of these events contribute to brain tissue damage. Stroke involves a complex pathogenic cascade of events, which includes energy depletion, excitotoxicity, acidosis, peri-infarct

depolarization, neuroinflammation, and oxidative stress. Thus, the ideal treatment might need to make use of combined therapies (Dekanski et al., 2011; Radenovic et al., 2014).

In parallel, oxidative stress is considered to play a significant role in the onset and progression of AD. Transient hypoxia in AD can lead to mitochondrial dysfunction, impaired membrane integrity, and APP cleavage. During the progression of AD, lipid peroxidation, protein oxidation, and DNA oxidation have been reported (Hayashi et al., 2006). One of the major pathogens of AD - A β , can lead to inflammatory cell injury through a variety of routes. A β can not only precipitate a significant inflammatory response with microglial activation and the secretion of TNF- α , but also A β can elicit the neuronal expression of inducible NO-synthase, peroxynitrite production, and neuronal apoptosis during an acute inflammatory response. The generation of oxidative stress by microglia during A β deposition suggests that microglia play an important role in the pathogenesis of AD (Hayashi et al., 2006).

The possible therapeutic targets should be in connection with the common underlying pathophysiological mechanisms in stroke and AD, such as excitotoxicity, oxidative stress, neuroinflammation, and apoptosis. Thus, new and creative approaches are needed. In that view, natural products of diverse structures and pharmacological activities from marine organisms, were tested in stroke and AD models (Choi et al., 2015). It has also been shown that sphingolipids can act as bioactive molecules that control diverse cellular processes, such as proliferation, differentiation, growth, migration and apoptosis, and thus could be used in pre-clinical studies for the treatment of pathophysiological mechanisms underlying neurodegenerative diseases. Emerging evidence reveals that sphingomyelinase/ceramide pathway plays an important role in neurodegenerative diseases that involve mitochondrial dysfunction, oxidative stress and apoptosis (Chen et al., 2012). Minocycline, a semi-synthetic second-generation tetracycline derivative in clinical use for infection control, is also considered as an effective protective agent in various neurodegenerative diseases in pre-clinical studies. Since it acts *via* multiple mechanisms including anti-inflammatory, anti-oxidative and anti-apoptotic effects, minocycline is a desirable candidate for clinical trials in stroke and AD (Chen et al., 2012).

Several studies indicated that natural venom components can exhibit selectivity and affinity for a variety of targets in mammalian systems. For instance, natural peptides identified in venoms from animals such as snakes, scorpions, bees, and spiders, were shown to lessen inflammation, regulate glutamate release, modify neurotransmitter levels, block ion channel activation, decrease the number of protein aggregates, and increase the levels of neuroprotective factors (de Souza et al., 2018). Thus, these venom components have potential as therapeutic tools to slow neurodegeneration in post-stroke and AD brain. The diversity of the components in the venom of several animal species, as well as their high specificity to cell targets, contribute to making the pharmacological research of toxins a promising field (de Souza et al., 2018).

The limited capacity for neurogenesis in the human brain makes neurological conditions such as stroke and AD especially difficult to treat. Stem cell research has thus an important potential in the treatment of neurological diseases through cell replacement and stimulation of endogenous repair systems. However, there are still numerous questions that remain unanswered with regard to stem cell therapies including optimal cell type and dosage, mechanism of action, route of administration, long-term safety, etc. (Duncan and Valenzuela, 2017). The efficacy of stem cell therapies is also related to the extent to which the cells exhibit trophic factors, and other secreted therapeutic molecules (e.g., anti-inflammatory

chemokines and cytokines). While substantial progress has been made in neuroregenerative stem cell therapies, further investigation is needed to optimize them for clinical use (Tajiri et al., 2014).

Advances in induced pluripotent stem cell (iPSC) technology have the potential for development of so-called disease-in-a-dish personalised models to study disease mechanisms such as stroke and AD, and reveal new therapeutic approaches (Nair et al., 2017). Different cell types can also be produced for therapeutic use. In 2015, the US Food and Drug Administration granted new investigational drug approval for the first phase 2A clinical trial of ischemia-tolerant mesenchymal stem cells to treat AD. Similar trials are either underway or are being planned in Europe and Asia. Although safety and ethical concerns remain, there is a need for the acceleration of human stem cell-based translational research into potential treatments of AD (Hunsberger et al., 2016). The induction of functional subtype-specific neurons of the basal forebrain from endogenous non-neuronal cells by *in vivo* transdifferentiation might offer a novel mechanism for the reconstruction of degenerated neuronal circuits in AD (Smith et al., 2017).

Post-stroke acute brain injury typically peaks within 24 h of the insult, and reaches completion within 48 h. Due to the quick onset and short duration of acute brain injuries, potential neuroprotective therapies need to be administered early within 3–6 h of onset. This has proven to be challenging in the clinical setting. Any treatment outside of the 48 h window will offer no protection, and could instead be mainly restorative, targeting angiogenesis, neurogenesis, and synaptogenesis (Tajiri et al., 2014).

Finding a therapeutic approach that would delay the progressive secondary neurodegeneration will benefit stroke survivors. Hence, stem cell-based therapy has emerged as a potential treatment. To date, most cell transplantation studies have been conducted on animals during acute phase of post-stroke injury, leaving chronic time points understudied. Preclinical studies have demonstrated safety and efficacy of cell therapy in animal stroke models, while limited clinical trials provided solid safety and tolerability in stroke patients transplanted with human adult stem cells (bone marrow stromal cells; Acosta et al., 2015).

Recent advances in cell identity reprogramming have enabled investigation of the mechanisms of neuronal degeneration, as well as the mechanisms that govern age-related neurodegenerative disorders. Being early-stage biomedical technologies, stem cell-based reprogramming and transdifferentiation each face numerous challenges that limit clinical utility. As these limitations are overcome through refinement and innovation, diverse safe and efficient reprogramming methodologies will emerge to establish a new paradigm for patient-specific treatment of brain disorders such as stroke and AD (Smith et al., 2017).

In conclusion, new and creative approaches are needed for possible therapeutic targets, for stimulation of endogenous neurogenesis and synaptogenesis, as well as for exogenous neuroreplacement in stroke and AD.

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Chapter 109

VIDEO-BASED QUANTIFICATION OF PATIENT'S COMPLIANCE DURING POST-STROKE VIRTUAL REALITY REHABILITATION

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ABSTRACT

We present a video-based monitoring system for the quantification of a patient's attention to visual feedback during robot assisted gait rehabilitation. The patient's face and facial features are detected online and used to estimate the approximate gaze direction. This gaze information is then used to calculate various metrics of the patient's attention. Results demonstrate that such unobtrusive video-based gaze tracking is feasible and that it can be used to support the assessment of patient's compliance with the rehabilitation therapy.

Keywords: video-based attention monitoring, gaze estimation, stroke rehabilitation, user compliance

INTRODUCTION

Stroke is the third most common cause of death in Western society, with about 4.7 million stroke survivors alive today. One of the hallmark residual impairments of stroke is

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post-stroke walking disability, which creates a stigma for patients, makes them more susceptible to injury and directly affects their quality of life. Early rehabilitation therapy is crucial for significant improvements (1). In recent years, robotic systems have been widely tested and employed to retrain stroke patients. By imposing gait-like movements at a comfortable speed, such robotic devices are thought to provide many of the afferent cues regarded as critical to the retraining of locomotion (2).

However, a major problem with existing stroke therapies is patient non-compliance (3). Many stroke patients abandon the therapy because the process is too long, repetitive, and/or does not provide immediate results (4). The European project BETTER (5) recently addressed a new approach to gait rehabilitation by employing non-invasive brain-neural computer interaction (BNCI) based assistive technologies based on EEG (Electroencephalography), EMG (Electromyography) and IMU (Inertial Measurement Unit) sensors. In this article, we extend the BNCI-based modalities with a video-based attention monitoring system that allows automatic quantification and long-term monitoring of user attention. Such attention tracking does not require any sensors to be attached to the patient, making this method easy and fast to apply in stroke rehabilitation. Our main objective is to quantify the patient's attention to visual feedback, i.e., the amount of time the patient's gaze is actively following the displayed visual feedback and to inspect the possible impact of visual feedback on motor planning, as well as its short- and mid-term benefits.

Several studies have already examined the impact of visual feedback on stroke rehabilitation, but they mostly reported inconclusive results. They focused on quantification of results of rehabilitation, enhanced with different kinds of visual feedback, but their experimental designs did not allow for a reliable quantification of the attention a person is paying to stimuli. To the best of our knowledge, only psycho-physiological measures of a patient's attention to visual feedback has been reported in (6), while video-based assessment of attention has not been proposed in the field of rehabilitation.

Methods for real-time detection of the face, facial features, eye movements and gaze direction from video have attracted a lot of research in the past decade (7). Numerous algorithms for facial feature extraction have been proposed, mostly in the context of face detection and recognition (8). Currently, the most promising approaches rely on fusing multiple visual cues, such as combining local feature matching with intensity-based methods (9). Existing methods for the quantification of user attention to visual feedback have mostly been developed for Human-Computer Interaction applications and in order to help severely disabled people (10).

METHODS

An overview of our approach is depicted in Figure 1. The patient is fixed in a robotic gait trainer, which moves the patient's legs according to predefined rehabilitation scenarios, and is adapted to the patient's current walking abilities. In front of the patient is a large TV screen showing various types of visual feedback. A high speed video camera is mounted above the TV. The camera captures HD video of the patient's face, while simultaneously EEG data are recorded from 51 scalp sites and EMG data are recorded from both legs (tibialis anterior muscle).

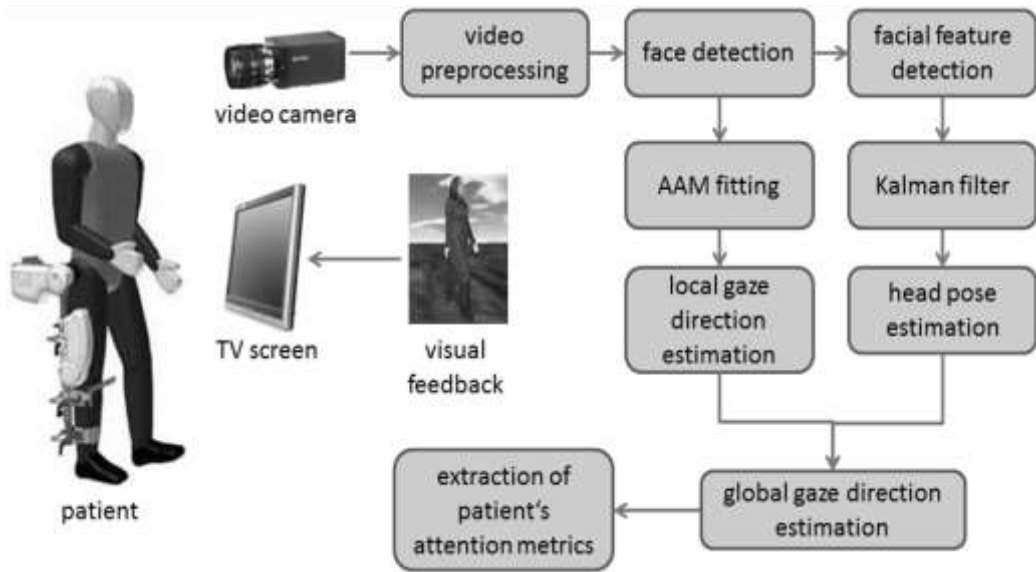


Figure 1. Overview of the proposed approach for video-based estimation of patient's attention/compliance during robot-enhanced rehabilitation.

The video streams are processed by our algorithms. First, frontal faces are detected and main facial features are extracted (corners of the eyes, pupil centers, mouth, tip of the nose). To suppress jitter from detection errors and body swings during walking, a Kalman filter is applied to the locations of the extracted facial features. Next, an active appearance model (AAM (11)) of the face with 59 facial landmarks is used to more accurately represent the current facial pose/expressions. The relative distances between extracted landmarks are used to calculate the head pose and gaze direction. Finally, different metrics of the patient's attention/compliance are computed: the percentage of time the patient is observing the visual feedback, the spatial gaze distribution maps, the responses to actions in the virtual reality (VR) environment, etc.

We tested five different types of visual feedback (see Figure 2): a calibration screen for the initialization of our algorithms; 2D plots of current leg positions; 2D plots of muscle activations; and two realistic VR environments (walking in a park from 1st and 3rd person perspectives). The VR environments were created in OpenGL 3.3 and consist of ground, sky dome and male/female avatars. The avatars were created in Mixamo Pro Character Creator Tool and animated with AutoDesk 3DS Max 2012. Both avatars have an underlying bone structure with all major joints modeled, allowing animation of almost all human movements. The actual avatar movement within the VR environment is controlled by kinematic data from the robotic trainer, so leg movements in VR world correspond with leg movements in real life.

The whole video processing system was designed for real-time operation, but the current version of the software is a mix of C++ and Matlab routines and runs at approximately 1 frame per second. Therefore, all video processing is currently performed offline, but (near) real-time operation is possible with further optimization of the code.

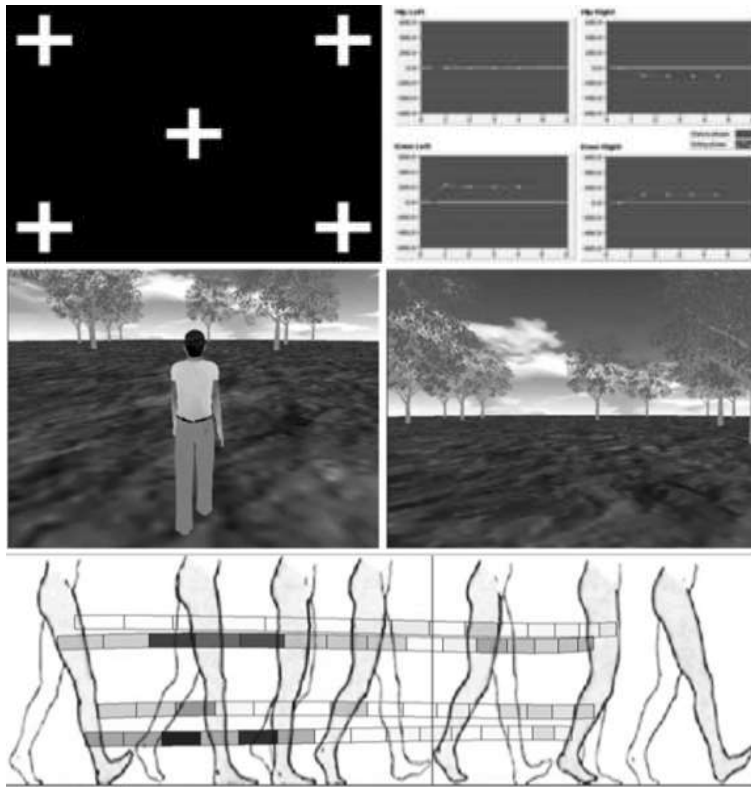


Figure 2. Five different types of visual feedback that are shown to the patient during therapy. Top row: the calibration screen for gaze direction estimation, and gait trainer's plots of current leg positions. Middle row: realistic VR environment from 1st and 3rd person perspective. Bottom row: special feedback displaying muscle activations.

EXPERIMENTAL RESULTS

The proposed system was evaluated in an experiment that involved 4 stroke patients and consisted of 5 runs of robot assisted walking with different forms of visual feedback. In all runs, the patients were instructed to walk actively, maintaining a constant speed, and applying minimum force on the robot. A 42" screen was placed in front of the patient, 1.4 m away from his face. Each run lasted 4 minutes. For all types of visual feedback the walking speed remained constant. During the experiments videos of the patient's face were recorded to disk in HD resolution and later processed offline.

On average, the face was detected in 93% of video frames recorded (i.e., in practically all the frames with frontal faces whereas the profile faces were not detected). Eyes, mouth, nose, and pupils were accurately detected in more than 99% of detected faces (see Table 1). Detection of facial features was skipped in video frames where no face was detected. The average jitter of detected facial features was 1.0 ± 0.4 pixels. The AAM was successfully fitted to all frontal faces. The average jitter of AAM landmarks was estimated at 0.6 ± 0.4 pixels.

Table 1. Facial feature detection performance, estimated on three test videos

	Video 1 (gaze targets)		Video 2 (gaze targets)		Video 3 (VR 3 rd person)	
	Frames	%	Frames	%	Frames	%
Whole video	9999	100%	13391	100%	10551	100%
Face detection	9833	93.7%	13193	98.5%	10427	98.8%
Detection of left eye	9805	99.7%*	13174	99.9%*	10419	99.9%*
Detection of right eye	9769	99.3%*	13179	99.9%*	10408	99.8%*
Detection of left pupil	9800	99.9%#	13191	99.9%#	10419	100%#
Detection of right pupil	9769	100%#	13187	100%#	10408	100%#
Detection of nose	9827	99.9%*	13150	99.8%*	10423	99.9%*
Detection of mouth	9742	99.1%*	13174	100%*	10414	99.8%*

* Values normalized by the number of face detections.

Values normalized by the number of eye detections.

Videos recorded during sessions with the screen displaying calibration targets were inspected by an expert and 9 approximate gaze directions (top-left, top-centre, top-right, bottom-left, bottom-centre, bottom right, left-centre, right-centre and centre of the screen) were manually annotated. The time periods corresponding to eye movements or eye blinks were ignored and were not annotated. Gaze direction was then calculated automatically by our algorithm and compared to the manually annotated gaze directions. The gaze directions were identified with average accuracy of $94\% \pm 6\%$. Most errors originated from distinguishing between top-left vs. bottom-left and top-right vs. bottom-right gaze directions.

Figure 3 shows an example of a patient’s detected level of attention to visual feedback, estimated as percentage of time, in 1 second intervals, with gaze fixed to the TV screen; all gazes outside the screen were classified as non-attention. Figure 4 presents an example of the estimated spatiotemporal gaze distribution metric. This metric shows relative frequency of identified gaze locations over a 4 minute long gait rehabilitation session. Brighter spots in the gaze distribution plots indicate areas with more frequent attention. Such maps support the identification of gaze targets (i.e., gaze hot-spots) and the assessment of spatiotemporal correlation between a patient’s attention to visual feedback and other BNCI-based performance indices of gait rehabilitation.

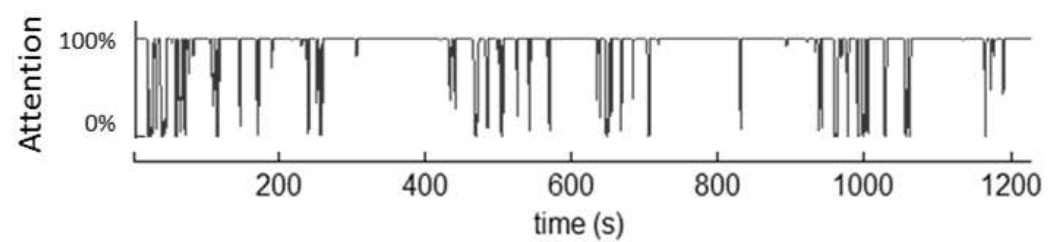


Figure 3. An example of estimated level of a patient’s attention to visual feedback during a 20 minutes long rehabilitation session (5×4 minutes).

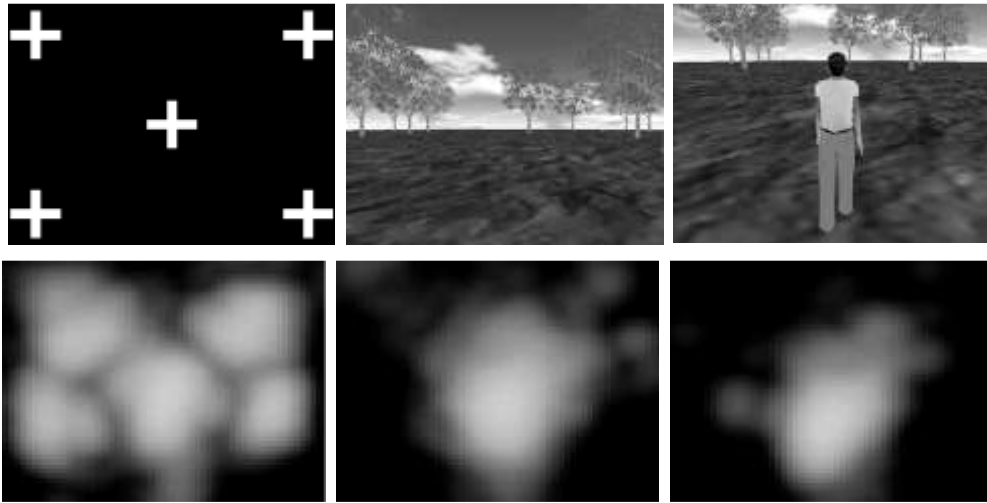


Figure 4. An example of spatial gaze distribution plots (bottom row) for three different types of visual feedback (top row) shown to a patient during 4 minutes long gait rehabilitation sessions.

CONCLUSION

We developed and validated a video-based system for the robust tracking of a patient's face pose and gaze direction during robot-assisted lower limb rehabilitation therapy. The results (see Figures 3 and 4) show that such a system can be used to unobtrusively analyze patient's attention to displayed visual feedback and to provide a means for long-term quantification of visual feedback effects on the rehabilitation progress. It can also serve as an additional feature for other BNCI performance indices, such as similarity of motor modules, kinetic and kinematic profiles and brain patterns. For example, preliminary results show a significant correlation between attention to muscle activation plots and improvements in muscle activations during walking.

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Chapter 110

COLOUR-CHECK IN STROKE-REHABILITATION GAMES

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ABSTRACT

This article presents the colorimetric testing of rehabilitation games designed for the StrokeBack project. In this testing, the main subject of the investigation was how the people with different colour-blindness types can perceive the games. Many programmers and game designers do not pay attention to the accessible aspect of games. This accessibility implies that users who are colour-blind should be able to use the games the same way as the people with no vision impairment.

Keywords: colour-check, rehabilitation game, stroke

INTRODUCTION

The purpose of the research described here is to investigate the colour palette of virtual reality (VR) based rehabilitation games (1), as designed for the ‘StrokeBack’ (2) project. The goal of the StrokeBack project is to improve the speed and quality of stroke recovery through the development of a telemedicine system which supports ambulant rehabilitation in home settings for stroke patients with minimal human intervention. During our investigation we designed our VR based rehabilitation games in such a way that they would also be enjoyable for people who have colour-blindness.

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The disorders in colour vision can be inherited and acquired. The cones' red and green colour specific photoreceptor cells' genes are linked to the X chromosome. Because of the gender-linked inheritance, this type of impairment is 20 times more prevalent in men. About 8% of Caucasian males and 0.4–0.5% of females are “red-green” colour-blind. Inherited blue-blindness (tritanopia) is much rarer – only about 0.05% of the population can be detected. Some colour vision disorders are not inherited, they are so called “acquired”; several ophthalmological diseases can result in colour perception disorders (e.g., retinal diseases, glaucoma, cataracts, etc.) (3).

Stroke is a leading cause of death in the United States, killing nearly 130,000 Americans each year – that is 1 of every 20 deaths (4). Every year, about 800,000 people in the United States have a stroke. About 610,000 of these are first or new strokes; 185,000 are recurrent strokes. Evidence from opticians indicates that about 10% of the male population are colour-blind. This, together with stroke incidence rate, leads to a statistically important problem.

STATE OF THE ART

The harmony of colours and proportions play an essential role in the appearance. Although many tend to neglect these issues, numerous scientific research studies are concerned with this field. All this is based on the colour perception of the eye, which, however unconsciously, may have an influence on patients' decisions.

Scientists and artists over the last centuries (5–7) and nowadays (8) developed colour order systems where they defined rules to establish harmonic sets of colours. These colour harmony studies were based on the orderly arrangement of colours in the colour order system. The second group of authors (9, 10) defined colour harmony as an interrelationship of colours. The main principles of these studies are “complementary” and “analogous” but these concepts are not consistent among the studies. Also, the colour wheel was often adopted as a tool to define these basic relationships. Judd and Wyszecki (11) define colour harmony as a more universal concept: “when two or more colours seen in neighbouring areas produce a pleasing effect, they are said to produce colour harmony.” In addition, there is no consistency among the principles and the keywords of colour harmony: it is *completeness* according to Goethe (9), *order* according to Nemcsics (8) and Chevreul (10), and *balance* according to Munsell (6). A quantitative model for two-colour combinations based on the CIECAM02 colour appearance model was developed by Szabó et al. (12). Many other effects (i.e., age, cultural background) of colour harmony feeling have yet to be investigated.

In visual experiences, harmony is something that is pleasing to the eye. It engages the viewer and it creates an inner sense of order, a balance in the visual experience. When something is not harmonious, it is chaotic. At one extreme is a visual experience that is so bland that the viewer is not engaged. The human brain will reject under-stimulating information. The human brain rejects what it cannot organize, what it cannot understand. The visual task requires that we present a logical structure. Colour harmony delivers visual interest and a sense of order. Extreme unity leads to under-stimulation, extreme complexity leads to over-stimulation. Harmony is a dynamic equilibrium (13).

Colour deficiency is often neglected, as most people do not consider colour deficiency as a serious problem. Up to 15% of the population being affected by one form or another of

colour deficiency (13). It is quite common to see combinations of background and foreground colours that make web-pages, software, games etc. virtually unreadable for colour deficient users. Background, text, and graphics colours should be carefully chosen to allow understanding for people with colour deficiency. Designing for colour deficient people is complicated. It is not simply a matter of green/red or yellow/blue combinations (13).

The most important issue in designing for colour deficient users is not to rely on colour alone to convey information and not to use colour as a primary means to impart information (14).

METHODS

Currently 3 games are built in the StrokeBack project:

- Break the Bricks game: practices horizontal (right-left) wrist movements – the player's task is to control the object at the bottom of the monitor;
- Birdie game: practices vertical (up-down) wrist movements – the player's task is to make a little bird fly and not let it fall;
- Gardener game: practices finger extension movements – the player's task is to pump a virtual sprinkler to make the plants grow by watering them.

These games can be used during the home rehabilitation process by the patients or replacing/enhancing clinical rehabilitation, thereby speeding up the process of recovery. These games are single player games which the patient can play by himself/herself. It is expected that the patients will play more with the games than they would do exercises. To these games several 'locations' were developed:

- Break the Bricks: default bricks, car, cake, duck, ship, train.
- Birdie: default meadow, cave (played with a bat), circus, forest, jungle, mountain, sea, town, winter.
- Gardener: bluebell, carrot, currant, geranium, grape, pepper, rose, strawberry, tomato, tulip.



Figure 1. Gardener's "Bluebell" and "Grapes" themes as seen by a person with good vision.

Besides the 24 backgrounds for the 24 themes, more than 170 different objects had to be inserted, each of which had to be clearly visible. These were tested by different colour-blindness simulators and automatic testers.

Figure 1 shows two themes of Gardener as seen by a person with a good vision. Figure 2 presents the jungle theme of game Birdie and the objects that can be inserted as obstacles in the theme.



Figure 2. Jungle theme of “Birdie” game and the objects that can be inserted.

RESEARCH AND DEVELOPMENT

For the investigation four different colour-blindness simulators were used, which are accessible on the Internet and where pictures can be uploaded (15–17), and a downloadable software, ColorOracle (18), with which we could test the pictures appearing on the screen on the developer computer to find out how the users of different types of colour-blindness – deuteranopia, protanopia, tritanopia – see the colours.

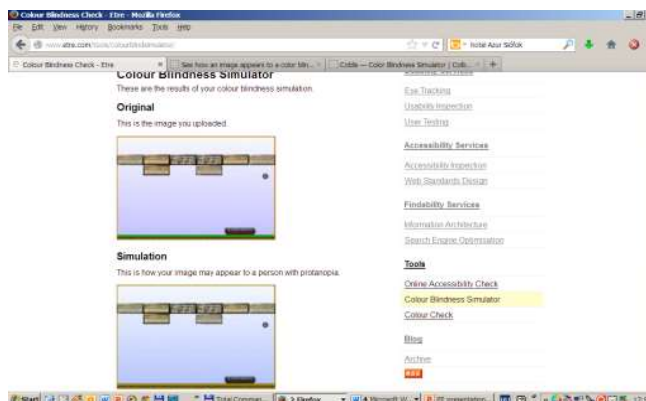




Figure 3. Testing of “BTB basic brick” theme with ETRE simulator (left) and “Birdie jungle” theme with ASP.NET based online simulator (right).

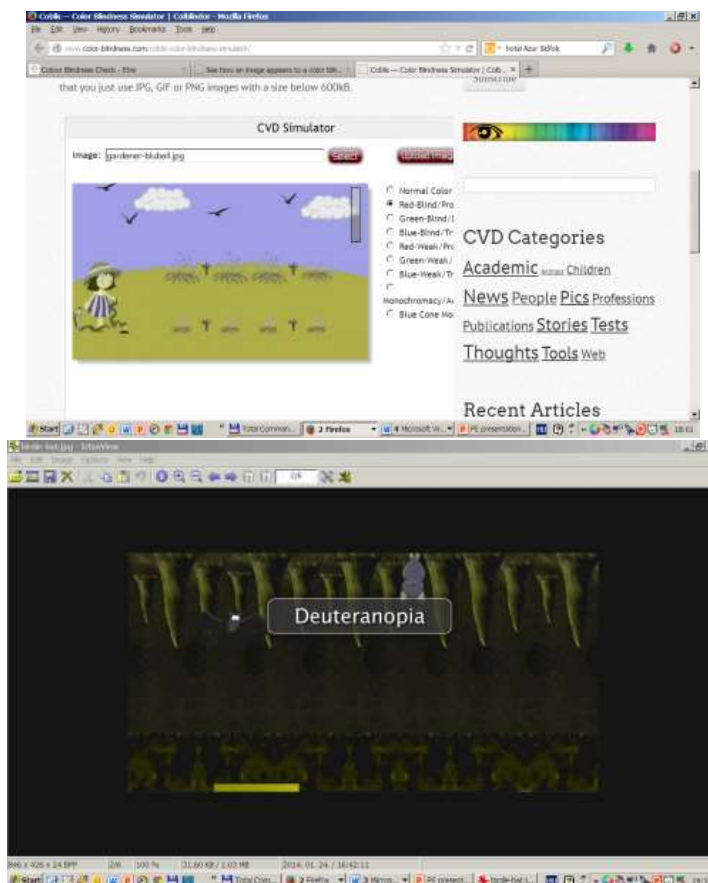


Figure 4. Testing of “Gardener” with Coblis simulator (left) and “Birdie cave” theme with ColorOracle tester.

Testing and contribution to the field

Testing has been completed not only with the *ETRE*-, *ASP.NET* based-, and *Coblis*-simulators and the *ColorOracle* tester, but also with Variantor's special glasses (19), with two individuals with good vision, and two persons with colour-blindness. Based on prevalence (10%) of colour-blindness in the male population, and the case that colour-blindness is aggravated by stroke (20), individual tests using a standard colour chart display has been conducted. Figure 5 shows the difference between normal colour vision (Figure 5a) and colour blindness in the red tone (Figure 5b).



Figure 5. Original colour chart (left). Colour chart vision with Variantor glasses (right).

The tests were completed by people with normal vision and those with colour-blindness. The visual elements of the games were not just tested with online applications. Out of the possible test subjects, we filtered for colour-blindness by applying the Farnsworth-Munsell 100 HueColor Vision test (21).

During the test, the subjects had to look at some parts of images taken from the games and were then asked questions regarding them. The questions concentrated on visibility and perceptibility, and the elements disturbing or obstructing visibility and perceptibility. Example questions are:

- *Jungle theme*: What kind of fruits could you see on the tree? How many? Which animal has a darker colour?
- *Cave theme*: How many bats have been in the cave? What objects could you see on the ground beneath the crab?
- *Forest theme*: Was there a wooden high-stand in the picture? What colour are the mushroom caps? How many separate clouds are visible?
- *Winter theme*: How many white animals could you see in the picture? From which direction did the bird (if there has been) fly to the image?
- *General questions*: What value did the achievement bar show on the right hand side?

Based on the test subjects' opinion, the achievement bar has been placed on the periphery of the screen and for the most of the time they have not even noticed that it is placed there. In the test subjects' judgement, the games' visibility and perceptibility is good, although they

have indicated that, in the circus theme, the white and red stripes of the circus tent have been disturbing, which generated the vibrating effect. In the case where the recurrence of some image elements have been questioned, the elements have been recognized in the pictures and the subjects have even been able to determine the correct number, but the colours of the elements could not be recalled. As an example, in the garden topic the subjects were able to tell how many tulips have there been on the picture, but they could not tell the colours of the flowers. Responses to questions on colour balance include remarks that the plants in the picture mostly fade into the greenish hue background and, in case of the jungle theme, the green parrot can hardly be noticed between the leaves of the tree.

For some of the users and test subjects the noisiness and pixel problems due to the low image resolution were disturbing. The test subjects have also emphasized some situations that had disconcerting effects, for example when combinations of colours have been shown which physically interrupted the focusing of the eyes, as in the case of combination red-blue colours.

The results of the tests have shown that the observers – despite their colour vision deficiency – have seen picture elements taken from the game appropriately, the small details having been recognized and identified. The separation, observation and recalling of the games' image elements did not cause any problems for the validly colour-blind test subjects.

CONCLUSION AND PLANNED ACTIVITIES

We have not found any publications that consider the testing of colours of rehabilitation games, lending credence to our view that developers do not think about the colour-blind people. As a result of our testing, we reached the conclusion that, within the StrokeBack games, the objects are clearly visible, so the colour-blind patients can practice in the same way as the normal sighted. In future, games should not only be tested with the software, but also with other simulation goggles (tunnel-vision, macula or lens degeneration). Clinical tests of efficiency (which refer to the colour testing and efficiency of the rehabilitation) were started in June, 2014, and future reporting will detail these results.

ACKNOWLEDGMENT

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Chapter 111

EVALUATION OF LEAP MOTION CONTROLLER AND OCULUS RIFT FOR VIRTUAL-REALITY-BASED UPPER LIMB STROKE REHABILITATION

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ABSTRACT

Intensive rehabilitation is important for stroke survivors but difficult to achieve due to limited access to rehabilitation therapy. We present a virtual reality rehabilitation system, Target Acquiring Exercise (TAGER), designed to supplement center-based rehabilitation therapy by providing engaging and personalized exercises. TAGER uses natural user interface devices, namely the Microsoft Kinect, Leap Motion and Myo armband, to track upper arm and body motion. Linear regression was applied to 3D user motion data using four popular forms of Fitts's law and each approach evaluated. While all four forms of Fitts's Law produced similar results and could model users adequately, it may be argued that a 3D tailored form provided the best fit. However, we propose that Fitts's Law may be more suitable as the basis of a more complex model to profile user performance. Evaluated by healthy users TAGER proved robust, with valuable lessons learned to inform future design. The majority of users enjoyed using the Leap Motion controller and VR Headset, the inclusion of visual cues shows a general improvement in target acquiring performance and that Fitts's Law can be used to linearly model user reaching and pointing movements in 3D environments. However, in some situations this

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isn't the case, emphasizing the importance of user profiling to examine the user's kinematic behavior more intricately.

Keywords: virtual reality, leap motion, oculus, stroke, rehabilitation, usability

INTRODUCTION

Upper limb rehabilitation following a brain injury, stroke, or other condition affecting upper limb movement, is carried out by occupational therapists and physiotherapists to recover and adapt the patient's movement and improve activities of daily living (ADL). Research confirms that rehabilitation is capable of improving arm function during both the early and late phases of stroke. However, effective therapy must be intense and requires the repetitive practice of task-related movements and actions (1). Repetitive task training involves intensive repeated practice of appropriate functional tasks and is thought to reduce muscle weakness and form a physiological basis of motor learning (2).

Virtual reality (VR) has significant potential to support self-management of such rehabilitation, in that it allows individuals to interact and train within stimulating, realistic virtual environments. It provides users with the opportunity to practice intensive repetition of meaningful task-related activities necessary for effective rehabilitation (3). A recent Cochrane review of 12 trials involving 397 participants for the upper limb, stated that the use of VR and interactive video gaming might be beneficial in improving upper limb function and ADLs; when used as an adjunct to usual care or when compared with the same dose of conventional therapy (4). This Cochrane review concluded that it is unclear at present which characteristics of VR are most important. However, they emphasized the need for pilot studies assessing usability and validity as part of the development process if designing new VR programs for rehabilitation purposes; these studies may also afford insight on the key VR characteristics for retraining of movement e.g., in reach and pointing tasks.

In this paper we expand on our latest research (5), presenting a multi-sensory VR system, Target Acquiring Exercise (TAGER), and evaluate its usability and performance for upper limb rehabilitation. We evaluate Fitts's law as the basis of an adaptive system to model movement performance for reach and touch tasks in a 3D virtual space. TAGER evolved from previous VR and augmented reality (AR) testbeds developed by our research group (6) and particularly from initial research undertaken with the Leap Motion controller (7). TAGER utilizes several natural user interface (NUI) devices to track the user, namely the Leap Motion, Microsoft Kinect V2, and the Myo armband, and affords the option of wearing a VR headset (in this experiment the Oculus Rift DK1). In the experiment outlined in this paper, the tasks comprise basic 3D reaching and pointing exercises so we can effectively evaluate the system and user model. The experiment reported here focused on testing with healthy users to help us evaluate the user interface ahead of planned experiments with impaired users, and has helped us refine the user profiling system. A participatory approach to system and experimental design was taken; engaging with physiotherapists and occupational therapists in local health trusts, namely the Regional Acquired Brain Injury Unit (Musgrave Hospital, Belfast), the Stroke Unit at the Royal Victoria Hospital (Belfast), and third sector agencies, e.g., Brain Injury Matters (Belfast).

A core interest in VR as a rehabilitation tool is due to its potential to provide an enjoyable experience. However, VR systems are flexible technologies supporting feedback, capability adaptation, high intensity, repetitive, functional exercises to encourage motor control and motor learning. There are an increasing number of studies mainly focused on using commercially available hardware devices to support upper limb rehabilitation (4). Recent cheaper, high-quality commercial technology, such as Leap Motion and the Myo, offer new opportunities for effective, center and home based self-managed rehabilitation. When combined with other existing technology, they have the potential to improve accuracy and reliability of performance monitoring, although research utilising these technologies is still limited. Feedback is of particular importance to patients, and VR systems have the potential to excel in providing rich, informative, personalized, just-in-time responsive feedback. Feedback cues in VR environments, when appropriately designed, can help users improve interaction performance. Rehabilitation systems have implemented multi-modal cues such as visual, tactile and auditory cues. The organization of movement is related to the quality of the viewing environment, particularly visual cues between the user's arm and the objects to improve spatial awareness (8). Tactile cues have also been reported to improve motor performance (9), and are mainly used to help users verify a successful interaction. Positive auditory cues provide user motivation to perform intensified repetitive tasks, represent temporal and spatial information very well and can improve motor learning (10).

Adaptation is an important usability factor within a VR rehabilitation user interface design, to enable a system to adapt both to a diversity of motor control capabilities and as rehabilitation progresses (6). This capability to adapt is important as a user may become frustrated if the tasks are too difficult or bored if tasks are too easy; thus maintaining engagement is vital in rehabilitation. Techniques proposed in the literature for adaptive VR rehabilitation systems include fuzzy logic and Fitts's Law (11). Fitts's Law is well known in the user interface community and has also been applied within the stroke rehabilitation (12) context. Fitts's Law models a user's motor skills by predicting the time to reach and touch a target based on a target's size (W) and the distance (D) from an origin (Eq. 1). The logarithmic element of the equation, known as the "Index of Difficulty" (ID), is used to quantify the difficulty of reaching a particular target. Thus, the movement time (MT) required to acquire a target is linearly dependent on ID , where smaller and further away objects are harder to attain.

$$MT = a + b \log_2 \left(2 \frac{D}{W} \right) \quad (1)$$

Equation 1 shows Fitts's original equation, but other researchers have devised variations of the equation to provide an improved model in different situations. Two popular adaptations of Fitts's Law are Shannon/McKenzie (eq. 2) and Welford (eq. 3), which were originally tailored to quantifying human movement behavior for 1D and 2D tasks. They have also been applied to 3D environments, but recently new forms of the equation have been devised to represent 3D interactive movements more accurately. Equation 4 (13) adapts Shannon/McKenzie (eq. 2) to include the addition of a movement direction parameter, to account for the consideration that MT is also dependent on the user's angle of motion (θ) on the Y axis from the origin to a target.

$$MT = a + b \log_2 \left(\frac{D}{W} + 1 \right) \quad (2)$$

$$MT = a + b \log_2 \left(\frac{D}{W} + 0.5 \right) \quad (3)$$

$$MT = a + b \log_2 \left(\frac{D}{W} + 1 \right) + \sin \theta \quad (4)$$

METHODS

The research described in this paper has an exploratory emphasis and focuses on investigating VR NUI design, particularly (i) the reliability of tracking systems and the modelling of user motion via Fitts's Law and its variants, (ii) the aesthetic design of multi-modal cues, and (iii) the usability and acceptability of the VR headset. The purpose of conducting this research – and the subsequent follow up with impaired users – is to ensure that the underlying interactive interface and adaptive motion tracking system is as robust as possible before adding further user interface elements, game components, and connected health systems. Ultimately our intention is that the system will be robust enough for unsupervised usage by stroke patients.

Participant recruitment

Healthy participants were enrolled in the experiment from students and staff at Ulster University. Inclusion criteria included that participants should be 18 years old and over, have no vision issues (e.g., blurred vision, color distortion, light sensitivity, depth perception), nor any disability that affected the upper extremity. Participants completed a questionnaire to gather information regarding IT and gaming literacy and to determine inclusion in the experiment.

Target acquiring exercise (TAGER)

TAGER is a custom designed 3D pointing exercise for upper limb rehabilitation, designed based on requirements from research (14) and clinician involvement. TAGER utilizes a number of technologies that work together to monitor and provide feedback to users while completing upper arm rehabilitation tasks. The Leap Motion controller is a small desktop NUI which contains an infrared camera specifically design to track fingers, hands and arms. It tracks up to 20 bones per hand, with 150° field of view and approximately 8ft³ of 3D interactive space. TAGER uses the Leap Motion as the main interactive device within the virtual world for tracking motion and facilitating target acquisition. Microsoft's Kinect V2 is similar to the Leap Motion, though instead of sensing hands it senses motion of the human skeleton. We collect data of all joints in the upper body in motion with the goal in a future version of providing guidance on suitable functional task movements and other factors. It is natural for a person with an affected limb to move their whole body forward rather than

extending their arm, especially if tired. As this would hinder improvement through the rehabilitation process, system feedback and guidance can be crucial. The Oculus Rift DK1 (VR Headset) contains a 7" screen with a resolution of 1280×800 (16:10 aspect ratio) and a 90° field of view and enables head positional and rotational tracking. User head motion controls the viewpoint within a virtual world. The Oculus is investigated in this experiment to determine if it is acceptable for use and could potentially be used to increase spatial awareness. The Myo armband slides onto a forearm, and uses electromyography (EMG) technology with eight medical grade EMG nodes attached, that read electrical signals from the muscles. The Myo armband also includes an accelerometer and gyroscope to track movement and orientation on the forearm. The use of the Myo armband here is to collect data to be stored for future studies helping identify changes in muscles, which could highlight factors such as fatigue and correctness of exercise. The Myo armband also includes tactile feedback capability, for example, vibrations on the skin, which are used to introduce tactile cues into the system.

Experimental setup

The experimental process comprised three stages: (i) a training stage which gives the participant ten minutes to familiarize themselves with the technology and practice interaction. The training tasks are very similar to the real trials. Through system testing and observing users before the actual experiment it was decided that ten minutes training would be sufficient time for the participant to familiarize themselves but not cause fatigue. After training, the patients are given a short two minutes' rest period before the next stage (ii), which is the complete TAGER trial. In stage 3, after each user completes the trial, a short discussion takes place gathering any comments the participant might have and for the investigator to ask questions related to the user's experience of the system. Throughout the experiment, the participant was closely monitored by the investigator who provided assistance if any problems arose. The experiment was strictly controlled; the location of each trial was always the same as was the equipment used. Equipment arrangement was identical for each patient ensuring no other possible variables could affect the collected results. Figure 1 illustrates the layout of the environment.

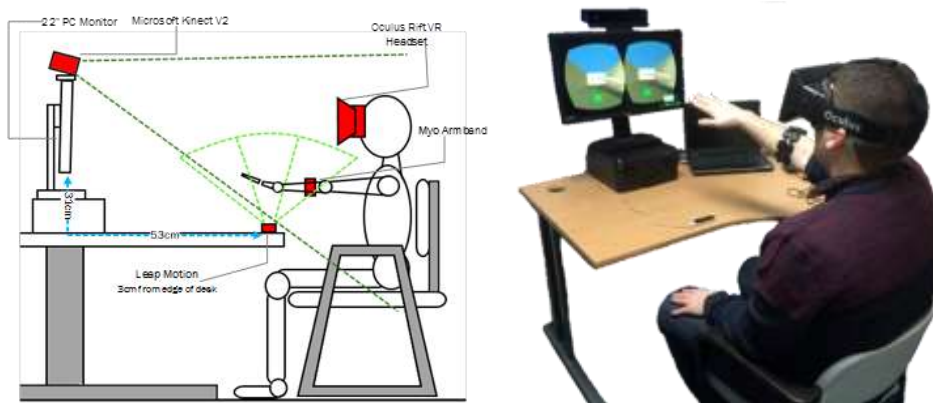


Figure 1. The Experiment setup showing the equipment locations and typically user interactions.

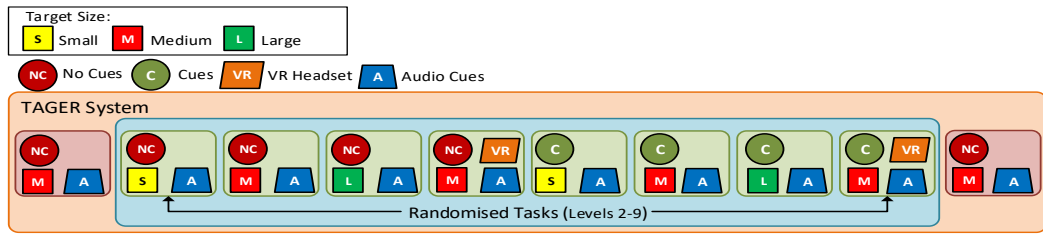


Figure 2. TAGER's level layout and scene attributes.

TAGER's 3D virtual environment is the inside of a basic walled room (no wall at the front) with a large start button on its floor. The user was prompted to push the button with their virtual hand to begin. An icosahedron shape was purposely chosen as the target object for its geometric properties, especially since visual cues are required to enhance spatial awareness. With changes in viewing angles, objects with a greater number of faces and edges such as icosahedrons, provide more clarity of visual cues (15). Each repetition (4 per level) contains 27 icosahedrons to target, all at different locations, a total of 108 per level (4×27). When the start button was pushed a single icosahedron appears randomly at any of the 27 locations. The user moves their virtual hand around the 3D environment touching each icosahedron that appears. When touched, the icosahedron disappeared, and another icosahedron appears on the floor of the room at the location of the start button. We call this object the origin; this approach was used to provide consistent movement trajectories. All objects were intentionally placed at fixed locations and at fixed distances from the user's view to simplify analysis.

The TAGER VR software for this research was constructed with ten levels; the first and last levels were identical enabling us to compare performance over the period of the session – considering learning and fatigue effects. All other levels were unique concerning scene attributes such as target object scale, multi-modal cues and VR headset use; to investigate the impact they had on user movement behavior (see Figure 2). Levels 2-9 were randomized per user to eliminate potential bias in the ordering; a rest period provided between each level and repetition. The unique levels comprise different combinations of scene attributes such as shadowing and proximity color change for visual cueing. Tactile cues were included using the Myo Armband where a vibration was sent to the user's arm upon successful target acquisition. We also changed the scale of the objects; objects were sized accordingly as 2, 3.5 and 5cm. Objects were scaled to discover the impact it has on cues. For example, larger objects were expected to give greater clarity to visual cues and thus quicker arm kinematics. These scene attributes helped build knowledge on the impact they have on arm kinematics, spatial awareness, movement speed and accuracy.

RESULTS

The above experiment was undertaken to investigate core aspects of TAGER. Data was recorded every tenth of a second from all input devices, and *MT* calculated and recorded to file automatically from tasks for analysis of the movement models. Of the 26 participants, three were excluded from data analysis due to missing data or system issues (loss of tracking).

Usability

Of the 26 participants, 77% reported that their experience using the VR headset was enjoyable, no motion sickness or other health-related effects were reported. 43% of people commented that they perceived their performance to have improved while wearing the headset, while 50% said that they needed time to adjust when first wearing the headset. We compared user performance between the VR headset and monitor use, with and without cues. We used a paired t-test to determine significant differences between users' average *MT* and found no significant difference between Cues ($T = 1.681$; $DOF = 21$; $p = 0.053$) or No Cues ($T = 1.591$; $DOF = 21$; $p = 0.063$). Though, there was a consistently slower user response when using the headset (Table 1). In other words, the participant's subjective experience was at odds with the objective measurement. Although this is not a significant issue so long as the effect is consistent among users, it is a consideration for future interaction design. Discussion with health professionals reinforced the importance of aesthetic cues in rehabilitation VR system design. We provided feedback on target proximity and acquisition through lighting and shading, as well as vibration from the Myo armband. Figure 3 shows variation in interaction difficulty with cues (C) or without cues (NC) for different sized objects (large – LRG, medium – MED and small – SML). While results were generally as expected – i.e., cues supported improved efficiency in target acquisition. It was not clear that cues improve performance for MED cue acquisition and more investigation is required. More detail will be covered on the effect of cues in the next sub-section.

**Table 1. The mean movement time comparing VR headset and PC monitor:
Cues(C), No cues (NC)**

Exercise	VR (NC)	Monitor (NC)	VR (C)	Monitor (C)
Mean MT (ms)	1174.2	1107.4	1165.0	1115.4
T-test P = (0.05)	0.063		0.053	

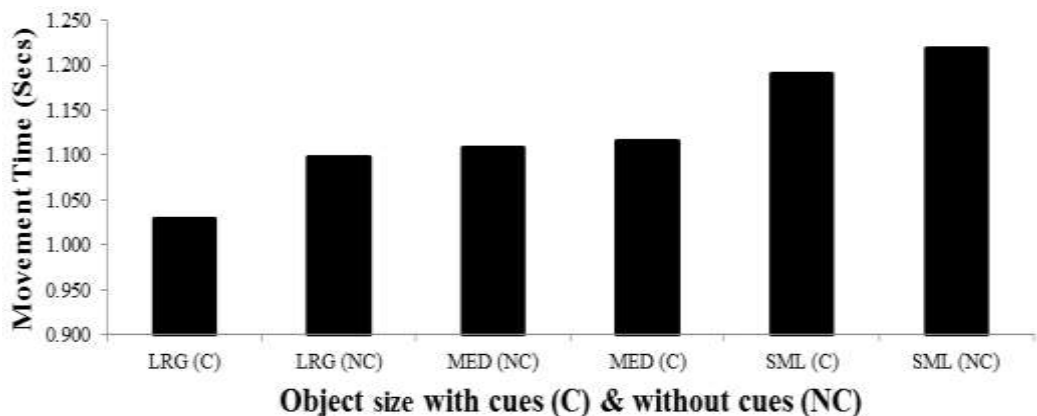


Figure 3. The variation of interaction difficulty arranged by the magnitude of the mean movement time.

An objective way to evaluate system feature effectiveness in TAGER is to record target acquisitions (hits) against movement times. A target has been successfully acquired when the user's hand collides with the surface of the target object without leaving the Leap Motion's detection area. Hits are classified as unsuccessful when the user's hand leaves the boundaries of the Leap Motion's detection area before hitting the target; users were required to return to within the boundaries to hit the target. Users could not progress until they acquired the current target, even after registering an unsuccessful hit. It was found that targets positioned at the center depth (relative to the user) were more successfully acquired – a mean of 29 successful hits per task over all participants (Front = 22, Back = 26). Closer objects appear to have been harder to attain, suggesting we may need to consider moving the minimum distance further away (along the z-axis) or move objects closer to the z-axis along the x and y-axes. The latter suggests a cone area (16) for object placement – with the cone pointing towards the user – rather than a cuboid (which maps well to the Leap Motion's detection area). This approach might account better for arm kinematic differences in attaining targets in close and far locations. Users attained targets in all sectors with reasonable success, though on average there were 31 (28%) unsuccessful acquisitions per level from a total of 108 targets. This may be considered to be quite high for healthy users; if the Leap Motion was mounted on the VR headset, changing position so that the leap camera is pointing forward (user's facing direction) could possibly provide a more natural interactive space, and reduce unsuccessful acquisitions. TAGER's experimental design implemented two identical levels one at the beginning and end of the experiment, enabling investigation of the potential learning effects indicated by improved performance or user fatigue resulting in performance decline. The mean completion times for all users for the start task was 1257.8ms while for end tasks the mean completion time was 1081.4ms suggesting that, despite having 10 minutes training time at the beginning, user performance significantly improved over the experiment ($T = 4.60$, $DOF = 21$, $p = 7.7E-05$). There are also some indications of fatigue (or loss of concentration) among several users. Analysis of experimental data in the next section enabled us to investigate the intricacy of user profiles.

Model effectiveness

As outlined earlier we recorded user motion data in reaching from an origin to touch an object target at various distances. Figure 4 shows the lines fitted to the data through regression for four well-known forms of Fitts's Law.

Table 2. The mean y-intercepts across each TAGER level for all forms of Fitts Law

EQ	SML (NC) (ms)	SML (C)	MED (NC)	MED (C)	LRG (NC)	LRG (C)	VR (NC)	VR (C)	Start	End	Overall Mean
(1)	189.3	77.4	270.7	161.6	392.7	343.4	70.6	-41.6	254.1	356.7	207.5 ± 89.3
(2)	-25.3	-152.2	95.9	-30.8	254.4	209.2	-107.9	-293.5	49.6	213.2	21.3 ± 110.9
(3)	86.4	-37.9	121.5	-7.5	210.2	166.6	-122.8	-250.8	74.7	224.4	46.5 ± 107.0
(4)	319.3	203.3	279.9	243.4	357.7	346.6	203.8	185.0	288.3	374.2	280.2 ± 83.6

Table 3. The mean slopes across each TAGER level for all forms of Fitts Law

EQ	SML (NC) (ms)	SML (C)	MED (NC)	MED (C)	LRG (NC)	LRG (C)	VR (NC)	VR (C)	Start	End	Overall Mean	Bits Per Sec
(1)	339.3	370.1	355.8	406.2	366.9	356.2	463.7	510.8	427.3	308.4	390.5 ± 46.0	2.5
(2)	315.7	343.2	316.6	358.4	311.0	301.9	392.7	454.3	378.1	271.6	344.4 ± 41.1	2.9
(3)	362.8	396.7	396.6	451.2	421.8	409.4	515.8	566.6	475.7	344.6	434.1 ± 51.2	2.3
(4)	247.7	273.1	275.1	290.5	282.1	261.7	318.5	324.1	322.9	236.2	283.2 ± 32.7	3.5

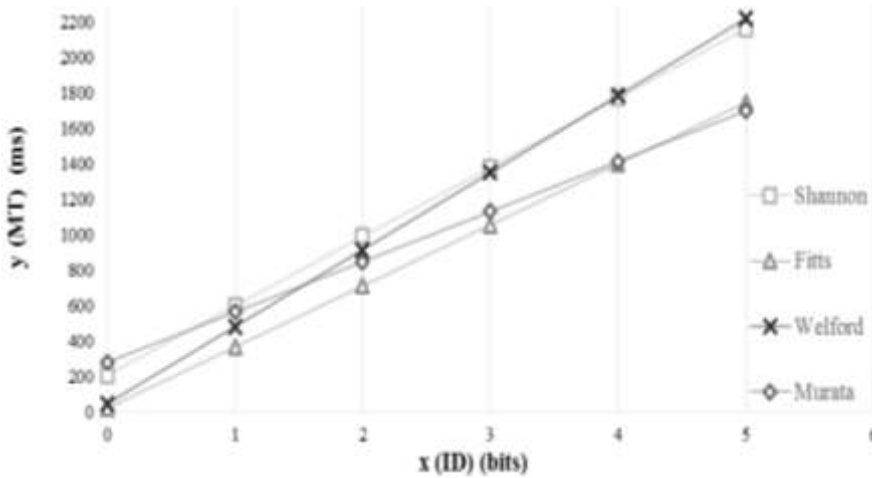


Figure 4. Regression line from our data for the four popular variants of Fitts Law.

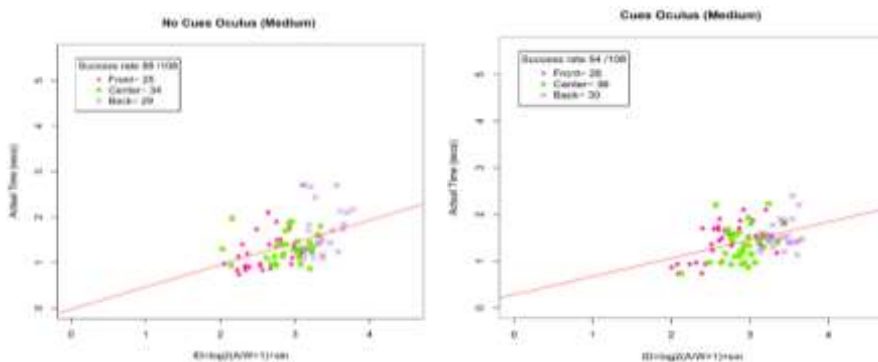


Figure 5. Murata's 3D model of movement for user 1023 while wearing the VR headset, plotting Index of Difficulty against the user's response time to successfully target medium sized objects. Depth (z-axis) is distinguished by 3 locations in the virtual world relative to the user: front (red diamonds), center (green squares), and back (purple crosses).

Tables 2 and 3 show the average regression values of the y-intercept and slope values for all participants. Murata's 3D equation (4) provides y-intercept values ranging from 196 to 363, which are more representative of human movement response (typically 200 to 300 ms); it also provided a more gradual gradient giving a more appropriate model of *MT* against *ID* (17). Thus, while all four equations provided similar results, we focused on Murata for further analysis. Figure 5 shows typical results while wearing the VR Headset with and without cues for medium sized targets and Murata's 3D form of Fitts's law.

Table 4. Participant statistics calculated over time including residual statistics, regression analysis and performance data to define a user performance profile

User	1001	1002	1009	1013	1019	1023	1026
Age : Gender	20 : F	20 : F	42 : M	24 : M	66 : F	54 : F	24 : F
Start (Residual)							
Standard Deviation	0.354	0.461	0.257	0.368	0.313	0.408	0.378
Kurtosis	0.085	-1.141	0.031	-0.238	-0.331	-0.262	0.376
Skewness	0.986	0.394	0.996	0.286	0.394	0.785	0.980
End (Residual)							
Standard Deviation	0.111	0.275	0.194	0.242	0.420	0.285	0.235
Kurtosis	5.852	0.985	2.540	-0.627	1.927	1.197	0.498
Skewness	1.834	1.227	1.515	0.187	0.952	1.143	1.289
Start Regression							
R ²	0.115	0.076	0.228	0.453	0.343	0.112	0.189
P-Value	4.05E-03	9.28E-02	8.60E-07	1.78E-08	1.79E-08	1.17E-03	3.92E-05
Slope	0.268	0.312	0.273	0.602	0.517	0.348	0.388
Intercept	0.352	0.360	0.268	-0.289	-0.212	0.475	-0.121
End Regression							
R ²	0.266	0.024	0.348	0.446	0.224	0.392	0.004
P-Value	3.80E-05	2.54E-01	2.83E-09	7.42E-09	3.18E-06	2.44E-11	5.77E-01
Slope	0.133	-0.109	0.293	0.445	0.481	0.509	0.033
Intercept	0.359	1.220	0.092	-0.070	0.116	-0.216	0.703
Performance							
Targets Hit (1080)	794	554	848	638	828	900	788
Start Hits	70	38	96	55	78	91	83
End Hits	73	57	85	59	88	92	74
% Change Hits	4.29	50.00	-11.46	7.27	12.82	1.10	-10.84
Start Mean Time	1.167	1.292	1.063	1.522	1.391	1.497	1.055
End Mean Time	0.768	0.884	0.952	1.270	1.580	1.262	0.802
% Change Mean Time	-34.19	-31.59	-10.42	-16.54	13.64	-15.66	-23.96

In Figure 5 when cues are included, user 1023 seems to improve her performance shown by a more gradual slope (decrease in value), the y-intercept (reaction time) towards a better representation of the typical human reaction time values and R² correlations are very similar. This experiment was fundamentally exploratory in nature. We used Fitts's law and linear regression to develop a user model, which will help us understand how best to create an adaptive system that can personalize interactive tasks. Table 4 provides user profiles from a proposed model comprising parameters such as regression line data, statistics on the residuals of the line fit, task performance and user information. Considering the explicit performance metrics (hits, movement times), target hits provided an informative statistic, and capable users are readily distinguishable from less able. Only one person had a score of less than 500 /1080 with the lowest score 472. The highest score was 900, and the average score was 754.74. Mean successful hits per person rose from 73.91 at the start to 77.22 at the end from a total of 108. The mean percentage improvement in hits over the experiment was 6.92% illustrating diversity of learning and fatigue effects among participants. The mean percentage reduction in MT was 13.79%. The average change in line fit according to R² and associated values were not significant (due to the spread of data values there are several valid lines). Change in hits

between the start and end tasks in the experiment were potentially revealing of a possible learning effect (improvement) or mental/physical fatigue. A learning effect provided a challenge to adapting task difficulty per user – it is preferable to adapt difficulty based on Fitts's law only after the learning effect stabilizes. Nonetheless, it was helpful to identify that a user remains in a learning phase so that additional support can be provided. However, MT and hits should not be considered independently, as some users may slow down deliberately to improve hit performance. The mean slope gradient decreased by 26.85% and the mean intercept decreased by 29.82%. Shallower slopes indicated a more skilled response as there is less difference between acquiring close and far objects, while a rise in the intercept value moves the intercept closer to physiological reflex norms (generally between 200 and 300ms). Additional user profile information can be gained from the residual statistics of regression lines and the changes between start and end tasks. Mean standard deviation dropped by 19.28% and mean variance also fell by 32.49%, suggesting that on average users were becoming more accurate. Range dropped by 10%, supporting this proposal. Kurtosis of residuals increased by over six times the magnitude (positive values) suggesting an increased number of data points with a small deviation from the regression line. On the other hand, skew also increased 44.4% (also positive), which after examining the regression graphs of users we believe was due to users overshooting the target position and having to change direction. From our results, we did not identify a strong relationship between performance and user age or gender.

Data from seven of the twenty-three participants provided a poor fit to the model based on start task motion data. Data after the end session from five participants had a poor regression fit, though two of these participants were different from the seven identified from the start data. These two people may have been overly tired or bored, rather than being less capable, highlighting the need for intricacy and careful analysis of the profiles. To consider this further it was necessary to examine a few representative individual user profiles (Table 4). User 1026 was an informative example, who started well with a strong profile but whose hits and mean MT declined significantly during the end task. Despite having residual statistics during the end task that show less variability (indicating more effective target acquisition for a significant number of targets), hit score actually decreased, while skew and kurtosis both increased (suggesting more overshooting of the target), and R^2 and the associated P-value suggested an unreliable regression fit (possibly due to an increase in outliers). 1026's mean MT dropped by a 23.96%, which suggested, considering the user profile, that during the end task this user may have adopted a high-risk strategy. This is in contrast to 1019, who appeared to have become more conservative, and by moving slower (13.64% increase in mean MT) improved their hit score by 12.82%. This considered approach by user 1019 and other participants seemed to improve the likelihood that the user motion data corresponds to Fitts's law (and variants). User 1002 had the weakest start having the lowest number of hits in the initial task but improved hit performance by 50% and mean MT by 31.59%. However, even by the end task, this user's motion could not be applied to Fitts's law reliably, and they had less than a 50% hit success rate in the final task. User 1023 exhibited arguably the most sustained success, having the highest overall hit success and start and end scores of 90 and 91 respectively and overall an improved profile (based on regression values). As with user 1019, 1023 was not particularly fast but improved speed during the experiment. Three participants got slower over the experiment and all of these achieved higher scores in doing so. Five participants got lower hit scores and all of these, but one had much less adequate regression

fit and three of these exhibited faster mean MTs. Further investigation is required to understand whether users with these profiles exhibit a decline of interest – the tasks were repetitive and several of these participants were quite skilled at the tasks – or due to mental/physical fatigue.

DISCUSSION

In this paper, we presented an initial version of the TAGER VR upper arm stroke rehabilitation system, which utilizes several inexpensive, commercially available input devices and a VR headset. We reported on an experiment that focused on the usability of the system and the use of various forms of Fitts's law to linearly model user motion - movement time against an index of difficulty – for reach and touch tasks in a 3D environment using a Leap Motion controller as an input device. Most users enjoyed using the Leap Motion device and perceived tasks to be easier with the VR headset on. No motion sickness or other negative health-related effects were reported. The importance of cues has been reported widely in the literature, and while our results show a general improvement in object acquisition with visual cues, we found the impact on targeting medium sized objects to be unclear. We outlined results from fitting user motion data to four forms of Fitts's law with each of the equations exhibiting a similar success. Examining the statistics of the regression process based on Fitts's law we found that these equations could be used to linearly model user motion with the Leap Motion controller in a range of setups including the utilization of a VR headset. However, the data point variance across the regressed line was quite high, and Fitts's law should not be applied as a simple linear model of user motion equally to all users. We recommend that a form of Fitts's law, for example, Murata's 3D version could be used as part of an intelligent system to profile users. Although the motion of most users could be modelled effectively using Fitts's law, we found a few users who found the NUI so difficult that they could not be modelled linearly. Most of these users required more training than we expected but in some cases, users appeared to become tired or bored. A more complex profiling system will help to identify training requirements and distinguish between loss of interest and mental or physical fatigue. We intend to develop the profiling system with further experiments and subsequently investigate the system with impaired users.

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Chapter 112

**REMOTE COMMUNICATION, EXAMINATION, AND
TRAINING IN STROKE, PARKINSON’S, AND COPD
CARE: WORK IN PROGRESS TESTING 3D CAMERA
AND MOVEMENT RECOGNITION TECHNOLOGIES
TOGETHER WITH NEW PATIENT-CENTERED
ICT SERVICES**

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ABSTRACT

This paper describes strategy and work in progress. The combination of patient centered care where many care and nursing units are collaborating with focus on, and in concordance with the patient, the ability to project information focused on the patient's total situation and needs independent where the information was created, the ability to use sensor technology to collect a wide range of aspects of the individual's health situation, the ability to use sensor technology to assess movements both for assessment and intervention purposes, to keep the care and nursing process together through module based information services and a structured care plan containing goals, subgoals, defined activity types and a wide range of health status data involve great opportunities for patients having chronic diseases. This group of patients causes extensive resource consumption for society. Well-structured data and semantic definition of data is a key for the ability to communication between different types of multi-professionals actors with different background. New technology like a wide range of sensor types allows the possibly to catch large amounts of data both for assessment and intervention purposes in

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a continuous way over time. This research group has worked on these issues for several years and some important milestones have been reached. From a chronic point of view three groups of patients are focused: stroke patients, COPD patients and patients with Parkinson's disease. Collaboration approaches, communication technology and adapted information services allow new ways to perform home based care. Integrated monitoring services of planned activities like motion activities using 3D sensors allows professionals and patient to in an exact way follow planned and executed motion activities which is of great importance to many patient needs.

Keywords: serious gaming, rehabilitation, patient centered care process

INTRODUCTION

A stroke or any other neurological disease/damage, like Parkinson's disease, has profound impacts on a person's life. The conditions are often life long and require continuous treatment and rehabilitation, as well as support for the activities of daily life. Communication and the performance of assessment activities as well as intervention activities are tiresome and it is a challenge to create a care situation with continuity for the patient in order to achieve results. Due to the low physical mobility and poor overall condition of these patients, traveling back and forth to doctors, nurses and rehabilitation centres can be exhausting tasks. Communication and interaction with relatives and friends is important but often become cumbersome and may also include long, strenuous trips. More developed forms of home based care, in combination with support from various kinds of professional units, has great potential for innovation, with information technology as an enabler.

Rehabilitation is essential in order to promote and maintain maximal level of recovery by pushing the bounds of physical, emotional and cognitive impairments. It is the foundation to enable full reintegration into the society and pursued occupation. Assessment is a key component in the care of the neurological patient and important for both diagnostic and therapeutic purposes in clinical practice. Patients often perceive their experiences of rehabilitation care as non-connected or non-coherent over time. Continuity and early start for the patient regarding rehabilitation is important to reach constructive results.

For COPD patients the need and importance of performing exercise is similar. Performing exercise can aim at keeping existing capabilities of the individual and to contribute to the avoidance of exacerbation.

For many patients home based care can be an effective way to manage rehabilitation without being required to visit the hospital or other care unit when training has to be performed on a more daily basis. Video mediated remote strategies can be used to support and encourage the individual in being systematic regarding exercises for assessment and intervention purposes. Monitoring can be performed both by observing the individual, using video, but new possibilities are developed by using monitoring services which in detail can monitor how different movements have been performed using worked out representation approaches of sub-movements of each exercise type. Monitoring and storage of detailed results of each exercise instance can be used for feedback, visualization and analysis both for the patient and for the professional.

Stroke, Parkinson's and COPD are examples of diagnoses where movements are of great importance both for assessment and intervention purposes and some exercises can be used for

several diagnoses (1, 2), but many types of exercises should be quite different depending on function resources and disabilities of the individual. For example, for stroke patients the rehabilitation service should allow exercises where differences between left and right parts of the body functions are focused.

In earlier work we have documented experiences of developing telemedical tools, tools for support of rehabilitation activities, including, sensor monitoring, 3D visualization and haptics (3-5).

Experiences of this earlier work lead to the insight that:

1. Information services is needed to keep track of the whole set of relevant conditions and goals of the individual to be able to tailor personal plans which can encourage the individual to perform evidence based care activities
2. Be able to perform more of self-managed care activities
3. Make it possible to add new types of sensors and to use the values for them in a structured way so that analysis and conclusions can be drawn related to accepted health condition types and classifications
4. Being able to point out affordable packages of equipment and services which can be affordable to most patients

Games are assumed to stimulate and reward a person, and to make it fun and engaging to perform serious activities. The aim is often and to reach results in terms of keeping up to a structured and individual exercise plan. If exercise activities can be performed through games a lot of encouraging effects can be made. A lot of development work is being performed to create a workable representation structure so that a good representation of exercise types (and its sub-movements) can be associated with one or several game type alternatives. The user can choose between different games for the purpose to perform certain types of evidence based exercises important for the individual.

For Parkinson's disease we are developing tools for remote assessment of motor function. The underlying scientific procedure is called Movement Disorder Society-Sponsored Revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (6): Process, Format, and Clinometric Testing Plan (7).

Recent work is focusing on monitoring, visualization and assessment of how the planned execution of the exercises really was executed. A specific monitoring part of the services has been developed that monitors and stores how every movement has been performed. This monitoring sub-service used the body representation and body posture to describe how every moment has been performed. Further, we have showed that depth cameras and other types of sensors are useful to follow movements (e.g., prescribed exercises) overtime and can provide a measure of rehabilitation progress in a wide range of remote health monitoring. The objective of this investigation is to develop tools and knowledge to identify if new technology matched with patient oriented services and new care procedures will lead to better rehabilitation in terms of both cost-effectiveness and quality for the individual. The overall goal is a multi-purpose, configurable ICT service platform supporting home based care highlighting the individual patient's specific needs.

Related to monitoring, one type of monitoring can consist of automatic collection of data concerning for example how an exercise has been performed, one other important type of monitoring is self-assessment by the individual him-/herself. For example, to COPD it is of

interest to collect data about how tiresome the performance of a particular exercise was experienced by the patient. For this purpose the Borg Scale can be used (8). Borg scale assessments must be linked to the exercise performance and to the elements of the sub-movements.

METHODS

3D sensors like the Kinect and other types of motion sensors like Leap Motion (LM) sensors are markerless motion capture systems which offer an attractive solution for home based rehabilitation (9). Although marker based tracking systems are more accurate, with spatial and temporal correspondence that marker less system lacks, the Kinect and LM devices' precision are sufficient enough for rehabilitation purposes (10, 11). The devices' accessibility and low cost render them an advantageous solution for home based rehabilitation. The video game environment provided by the sensors has the potential to become powerful motivation tools for performing regularly rehabilitation exercises. Data from tracking the execution of the exercises in real time can be used for assessment of patients' physical status, which can trigger the need for interventions. Furthermore, captured data can be utilized to provide guidelines to the user, thereby optimizing the effect of the exercise.

A promising approach, explored in some depth for stroke and COPD patients, is to integrate support for video-mediated communication into the rehabilitation support platform, which includes both LM and Kinect devices. The Kinect device is in this situation used both as a body tracking device and a camera, while the LM device is used for tracking of hand and finger movements. A therapist/doctor can thereby remotely assist or instruct the home cared patient, in cases where direct physical interaction is not needed. Related to patients with Parkinson's disease their motor performance will remotely be registered with the Kinect and LM sensors connected to a laptop or tablet computer. During the fall of 2013 we implemented one of the MDS-UPDRS procedure, namely "alternating movements" and tested it interactively with doctors and patients. Here the patient is instructed to repeatedly approach and remove the thumb and the index finger from each others as much and as fast as possible. For COPD patients there are similar challenges. One of the challenges is the importance of keeping up training and movements and repeatedly meet health care professionals for plan change and updates and also to meet relatives and friends to avoid depressions etc.

For several diagnoses, the risk of falls is significant and structured fall prevention activities are hence important. Many of the tools and technologies explored for Stroke, Parkinson's and COPD patients can also be used for fall prevention. Assessment, risk calculation and intervention activities can be designed to reduce the risk of falls, which can be beneficial for many kinds of home care.

RESULTS

From our previous experiences of stroke rehabilitation, we know that 3D sensor based approaches like Kinect works well in an interactive situation in assessing kinematic

information of motor function of the trunk, arm and leg. Now we have developed support for detection of hands and individual fingers. In most cases the interaction/instructions from the examiner can be pre-recorded 'machine' standards. Our preliminary study of the MDS-UPDRS indicates a drawback, namely that some tests must comprise a physical interaction or/and an, at the actual moment, individualized instructions from the therapist/doctor. In these cases, we have to develop alternative procedures or omit these parts. An added value, as compared to the clinically performed MDS-UPDRS, is that with the ICT tools we can assess kinematic, numerical, values specifying the tested functions. Examples are frequency of tremor, time/velocity/acceleration/precision in movements.

Further findings are that one has to structure the entire set of subservices to really take advantage of the technology potentials which can be used in the home. Development and tests have shown that the following parts are suitable:

- A component that can, in depth, represent a particular exercise type often consisting of a set of sub-movements. This includes variation variables for individual adaptation (see below),
- A component that allows the therapist to define which movement a patient should perform, including definition of regime, intensity, repetition, sets and adapted movements. This is part of the individual-ization and needs and goals for the individual,
- A component that can monitor and store how the patient actually is performing each particular movement at each particular occasion,
- A component that can represent all relevant health condition types and classification related to a set of diagnoses, over all goals, subgoals, goal values of health condition types and relevant relationships to activity types.
- A video-mediated communication tool designed for communication between a professional and a patient where the professionals really can see how different movements are performed in each planned session,
- A component that can describe game types and how they are related to evidence based exercise types.
- A follow-up module where results of each training session are visualized related to goals. This includes more exact monitoring of how each movement is performed by each body part monitored in real time.

To capture, store and analyze large amounts of data about how all exercises have been performed, approached based on Big Data analytics will be used. When monitoring every training instance, e.g., for COPD patients, it can be very fruitful to monitor and store all training instances including sub-movements and also simultaneously monitor medical sensor data such as blood oxygen saturation levels when performing a particular exercise. Based on the collected data, assessments can be performed and conclusions can be drawn about how that patient should perform certain types of exercises, resulting in an updated care plan.

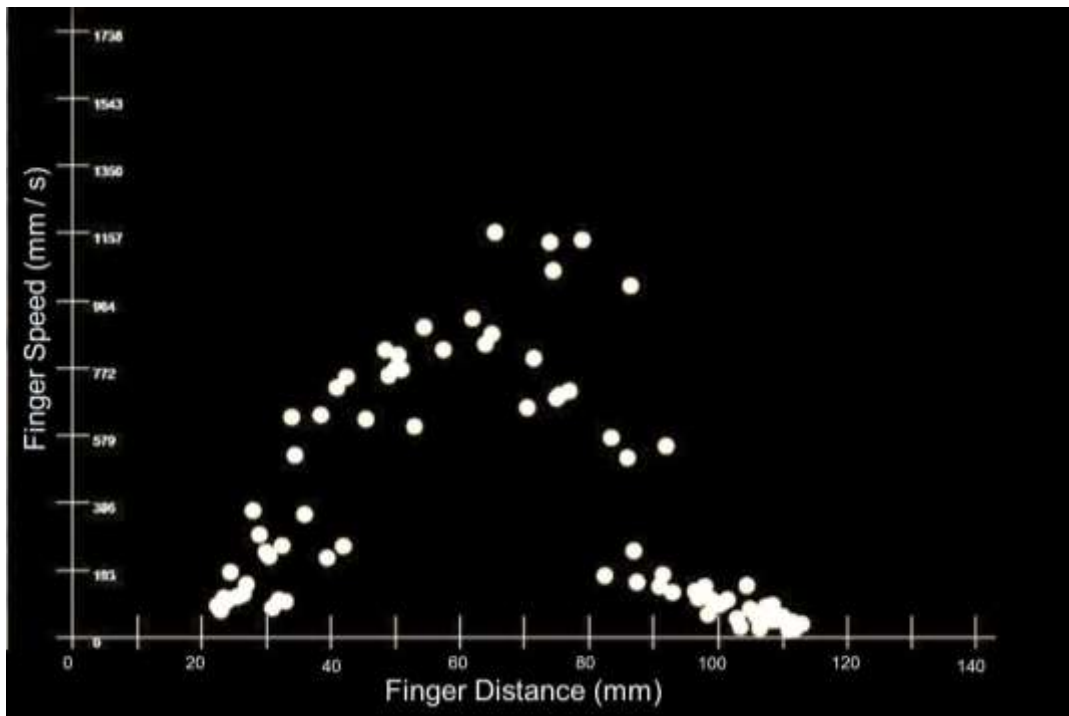


Figure 1. Shows the distance between the index finger and the thumb on the x-axis with respect to the relative speed between the two fingers on the y-axis.

The following plot (Figure 1) shows the distance between the index finger and the thumb on the x-axis with respect to the relative speed between the two fingers on the y-axis. It shows an arc like concentration of dots which is what it typically looks like for a person without any disorder. The more a person is affected the more irregular and flat like are the plotted set of dots. This measure is an indicator of dysdiadochokinesia, the inability to perform rapid alternating movements, a sign of cerebellar and/or frontal cerebral lobe dysfunction. This kind of assessment can be accomplished by having the Leap Motion sensor attached to any home computer and having the patient simply surf to a web URL where the test can be carried out.

DISCUSSION

The work is in a development and exploration phase but soon more systematic comparative studies will be performed in order to measure effects. Fundamentally, the health cooperation concept is cross organizational process where different care and nursing units and actors can participate. The focus is the patient's overall situation and needs. All actors involved in different types of activities can access selected information in a distributed unit, based on the roles that they have related to the individual. The individual and relatives are heavily involved in the performance of the activities.

The platform architecture has two main parts: the service level and the sensor level. A large number of new sensors have been developed and new sensors will appear. Sensors are typically interpreted in relation to an assessment scale, reflecting a specific health care aspect.

The service level is concerned with the logical process oriented interpretation, computation, visualization and storage of data. This architecture will continuously allow new technology to be adapted and integrated. All activities are structured into activity types and activity instances. Some activities are aimed at assessment of conditions and some activities are aimed at interventions related to goals (that change with time). The health plan is an important concept keeping all assessment activities, goals and intervention activities together. The plan can contain prevention as well as assessment and intervention activities.

In this patient/person centric approach, specific e-services, involving sensor technology, can be used for development of other movement oriented health assessment and intervention activities, like prevention of fall injuries and training of motor functions aimed at restoring muscle performance or breathing capacity. The e-services will contain subparts like definition of particular movement oriented activity types, support of how a particular activity instance should be executed, recognition of results of a particular activity instance, reporting of results of an activity instance, video-mediated communication with professional and other actors for support of a particular activity instance. For many activity types, including stroke assessment and rehabilitation, COPD rehabilitation and fall prevention activities, whole body tracking is the most important. For the UPDRS assessment activities, high precision finger tracking is also required.

The tool set outlined here can also contain tool parts supporting powerful assessment scales like the ICF scale. (The International Classification of Functioning, Disability and Health) (WHO) for the purpose of make an extensive analysis and assessment of the situation of the individual. This support can also contain support for setting of goal values related to ICF and the selection of activities for the individual.

CONCLUSION

Sensors, computers, displays and communication technology is getting better and cheaper enabling advanced e-services for home based care, including real time support from remote medical professionals. The developed solutions can be both cost effective and provide high quality for the patients, due to improved continuity of care, less need for travel, and improved motivation to perform physical activities more often. This means that e-health services for home based care, including communication with healthcare professionals, have a great potential to overcome the challenge with increasing demands of care due to an aging population. The services must support both asynchronous communication of patient data, as well as real time interactions for monitoring, assessment, and support. Not only health care personnel, but also relatives and other informal cares need to be included in the communication. By designing an ICT platform supporting both synchronous and asynchronous communication of data from tracking devices and other sensors, including depth cameras and video cameras, a flexible and configurable multi-purpose care solution for home cared patients can be developed. Until such a sophisticated platform is realized, however, experiments with enabling technologies in specific patient groups must be performed, which is the objective of our present study.

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Chapter 113

RADICAL APPROACHES TO STROKE

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ABSTRACT

Stroke and ensuing reperfusion injury is the third major cause of morbidity and mortality across the developed and developing nations. Neurological damage leads to cognitive impairment, a major deficit in behavioral, visual, sensory movement and muscle tone functions. In the absence of timely intervention, mortality seems to be the most common outcome of stroke incidence; however, restoration of blood flow does not guarantee complete recovery. Rehabilitation and post-injury management of stroke patient is the key to revive and ensure the normal functioning of the patient after stroke and reperfusion injury. Antioxidant supplements could enhance the chances of neurological and cognitive recovery while preventing further generation of free radicals responsible for cellular injury in massive stroke. Antioxidant actions are exhibited by agents obtained from an array of sources. This chapter shed light on various deleterious mechanisms of free radicals, its generation and available agents for restricting the cellular injury caused by the formation of reactive oxygen species (ROS) in stroke.

1. INTRODUCTION

Ischemic stroke is induced by permanent or transient obstruction of blood flow to any region of the brain which results in cellular and functional injury to cortex and hypothalamus. The obstruction of blood flow, in turn, results in deprivation of oxygen and glucose supply which instigate the cascade of major detrimental events responsible for cellular damage (Kalogeris et al., 2012; Carmichael et al., 2005). Similarly, restriction or obstruction of blood flow to any vital physiological organ lead to deprivation of oxygen and can result in severe

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ischemic condition. Restoration of the blood flow to this deprived region is the pre-requisite to restrict and revive the cells at risk (Choi and Pile-Spellman, 2018). Paradoxically, this delayed restoration of blood supply through occluded blood vessels lead to debilitating consequences responsible for instigating severe metabolic energy deficiency, inflammation and leading to permanent loss of functional cellular activity. The extensive cell injury due to this late replenishment of the ischemic tissue or organ is termed as reperfusion injury (Aronowski et al., 1997; Verma et al., 2002). The intensity of ischemic insult and the period of deprivation of nutrients to cells determine the reversible or irreversible nature of stroke injury. This cell death can be characterized microscopically by extensive swelling which may include detrimental anatomical changes in affected tissue, deformed protein band and hypertrophic mitochondrial structure (Piper et al., 2004).

Ischemic reperfusion injury can occur to any of the major organs in the body due to various circumstances. However, brain and heart seem to be the most vulnerable organs to encounter damage due to abnormal variations in blood supply. This blood flow restriction could be transient which closely resembles clinical situations of vasospasm in a coronary artery, resulting in angina, or cerebral hemorrhage resulting in mild to moderate cellular damage (Kloner and Jennings, 2001; Kloner and Jennings, 2001). However, with prolong and severe ischemia, extensive loss of functional ability with an array of pathological implications to organ's normal physiology, collectively called reperfusion injury (Yellon et al., 2000). Stroke or cerebral reperfusion injury occurs due to development of obstructions in a small vessel or large artery resulting in neuronal cell death could be associated with vascular endothelial damage or vasospasm, dysregulated homeostatic mechanism and abnormal hemodynamic activity (Kalogeris et al., 2012).

In most of the developed and developing countries, cardiovascular diseases and stroke account for nearly 2/3 of the total mortality and morbidity cases. Half of the estimated 32 million patients with acute vascular dysfunction are those having pre-established coronary heart diseases (CHD) or major cerebrovascular diseases (CVD) (Saposnik et al., 2009; Hallenbeck and Dutka, 1990). The risk of CHD and CVD recurrence is highest among pre-exposed patients with cardiac and cerebral ischemic episodes. Stroke can lead to recurrence in survivors at about 7% per annum, while mortality is fivefold higher in patients with recurrent myocardial infarction than those without any predisposition to CHD (Saposnik et al., 2009).

In the USA, stroke incidences are regarded as the third most relevant cause of death or disability across all population groups. The economic and social burden entrusted by stroke drains national coffers by the estimated annual expenditure of \$ 23.2 billion. The alarming mortality rate from stroke, more than 1,50,000 deaths per year, baffles researcher world over. The enormity of stroke-related social and economic implications had ushered extensive investigation of neuroprotective agents. The rehabilitation of the stroke patient is crucial for complete recovery. One estimate highlight the plight of stroke survivors as 76% of them end up being disabled for the rest of their life (Carmichael, 2005; Shi and Wardlaw, 2016). A large chunk of \$53.5 billion expenditure is incurred by the cost of supportive measures required for long-term stroke survivors. Thus, stroke disables more than it kills. These facts and figures gave an impetus to recent efforts to develop strategies for neural repair and rehabilitation after stroke (Carmichael, 2005).

Oxidative stress generated by ROS regulates basic mechanisms of cellular dysfunction that occur during ischemia/reperfusion injury (Granger et al., 2015; Kloner and Jennings, 2001). Ischemia-reperfusion injury induced ROS can damage almost all vital cellular

components, including cellular membranes to nucleic acids of the cells in contentions (Marczin et al., 2003). The agents scavenging oxidative stress can be of exogenous and endogenous origin and these compounds generally termed as antioxidants, have multifaceted activity including scavenging of ROS, curtailing ROS generation by chelating various ions involved this process. Various endogenous antioxidant supplementations found to be beneficial in restoring the antioxidant enzymes during reperfusion injury (Pizzino et al., 2017; Hill et al., 2011). However, the exogenous supplementation of lipoic acid (Dong et al., 2015; Cao and Philis, 1995; Shmonin et al., 2012), coenzyme Q10 (Liang et al., 2017; Lu et al., 2017) was found to be protective in the reperfusion injury induced animals.

Numerous reports highlighted the potential of antioxidants and their combinations with other neuroprotective agents on the reperfusion injury of brain and heart. The studies emphasizing the role of antioxidants in the management of stroke would further help to clarify the controversies associated with the exact mechanism of antioxidants and its clinical applicability in ischemia/reperfusion injury. Herein, the chapter explores the multifaceted mechanisms involved in ischemia and reperfusion injury in the brain. The fundamental ROS generating mechanisms, as well as the endogenous scavenging tools for limiting the oxidative stress injury, are discussed in details. This section also provides an overview of the therapeutic as well as experimental approaches available for the treatment of cerebral stroke and reperfusion injury.

2. PATHOPHYSIOLOGICAL CONDITION RESPONSIBLE FOR PROGRESSION OF STROKE AND CEREBRAL INJURY

Cerebral infarction may result from one or more pathophysiological mechanism, which includes atherosclerosis, cardioembolism, reduced systemic pressure, hematological disorder, and metabolic disorder. The diseases of large arteries and small vessels along with channel disorders can also play a vital role in the initiation of cerebral damage.

2.1. Atherosclerosis

A pathological condition where lipids deposits, generally calcified on the innermost intimal wall of the blood vessel which in progression hardens these arteries is termed as atherosclerosis. Plaques develop especially at bifurcations of major arteries including carotid artery. The biochemical processes ranging from fatty acid to formation of streaks leading to plaque formation due to excessive proliferation of smooth muscle cells. Moreover, an already hardened wall narrow down lumen and obstructs blood flow (Al Kasab et al., 2018). This obstructing of blood flow leads to turbulence in blood and increases kinetic energy, which dislodges the plaque in the bloodstream. Thus obstructed and narrower blood vessels restrict blood flow sometimes resulting in thrombus formation. Of all stroke cases, almost 33% cases have an association with atherosclerotic thrombosis (Kannel et al., 1971).

2.2. Cardioembolic Stroke

Cardiovascular dysfunction leads to ischemic stroke. The common disorders are familial arterial myxomas, hereditary conduction defects and hypertrophic obstructive cardiomyopathy (Hassan et al., 2000).

2.3. Reduced Systemic Pressure

Multiple mechanisms responsible for stroke and related complications arise from obstruction of blood flow in cerebral arteries leading to ischemic damage in and around the localized region (Alloubani et al., 2018). Turbulent blood flow weakens the walls of cerebral and cardiac vessels. This would greatly affect normal hemodynamic functions and result in altered systemic pressure among vital arteries adding to the factors responsible for the development of ischemia (Hademenos and Massoud, 1997).

2.4. Hematological Disorders

Several mechanisms are suggested for the increased risk of ischemic cerebral stroke in patients with sickle cell disease; these include sickling of red blood cells during a crisis and thrombotic infarction of both large and small arteries (Ilesanmi, 2010). Patients with sickle cell anemia are more prone to develop stroke as compared to others.

2.5. Small Vessel Disease

In CNS, blood supply to deep white matter and basal ganglia is obstructed due to the structural changes in the small penetrating end arterioles is the hallmark of small vessel disease. Lacunar stroke syndrome is the most common clinical phenotype of this malformation. These lacunar infarcts may lead to cognitive impairment and disorder of gait (Shi and Wardlaw, 2016).

2.6. Vasospasm

Spasticity of subarachnoid spaces due to sudden and excessive bleeding coupled with muscular contractions is known as cerebral vasospasm. The vasospasm may aggravate focal constriction leading to total occlusion (Wilkins, 1988). Any cerebral blood vessel encountering vasospasm, for the varied time period, induces cerebral ischemia.

3. TREATMENT STRATEGIES FOR ACUTE ISCHEMIC CONDITIONS

Increased understanding of the biological implications of ischemic injury led to the development of various treatment strategies to limit the extent of injury in the subject. The

primary objective of the treatment is to assure immediate survival of the patient and to restrict the damage in critical ischemic condition. However, the major limitation of the current development is lack of ability to prevent cell injury in the sudden ischemic milieu. Initiation of necrotic and apoptotic cell death in core and penumbra is contributing to the neurological deficit and impaired locomotors activity. The current drug discovery and development efforts need to focus on recovery as well as management of the ischemic cells and related physiological functions (Zheng et al., 2006).

3.1. Management of Acute Ischemic Stroke

Evident neurological deficit and a history of abrupt onset of symptoms in the absence of trauma suggest the onset of a stroke. Restoration of blood flow to threatened vulnerable tissue incidentally before it progress to infarction presents realistic opportunity to salvage penumbral tissue (Kalogeris et al., 2012). The reperfusion therapies significantly restrict infarct area and provide a window to limit cognitive deficit as well as bring a marked improvement in neurological outcome. General treatment includes the administration of supplemental oxygen tissue and isotonic intravenous fluid and anti-thrombotic/fibrinolytic therapy (Snow, 2016).

3.1.1. Intravenous Thrombolytics

Tenecteplase (TNK), a genetically modified form of tissue plasminogen activator (tPA) is highly selective for fibrin and prevents any inhibition of plasminogen activator type 1. The long lifetime of TNK allows it to administer in a single bolus dose and its specificity to fibrin ensures complete clot lysis (Snow, 2016). Presently, desmoteplase, abundantly available in the saliva of vampire bats, *Desmodus rotundus*, induces faster recanalization than tPA and is clinically evaluated in phase 2 trials of ischemic stroke (Molina and Saver, 2005). A recombinant peptide developed from human tPA consists of kringle 2 and protease domains of human tPA and works as a potent recanalizing agent and this recombinant tPA is marketed as reteplase. Similarly, antagonists of glycoprotein (GP) IIb/IIIa are shown to have the ability to improve flow by dissolving platelet-rich clots in coronary and cerebral microcirculation (Molina and Saver, 2005; Snow, 2016).

Intra-arterial thrombolytic therapy can be performed with a microcatheter that is placed into, beyond, and proximal to the arterial occlusion. Urokinase is conventionally used while intraarterial tPA and prourokinase have been extensively studied in recent investigations (Snow, 2016).

3.1.2. Combined Pharmacological Approach

The combination therapy is put forth to expand the fibrinolysis time beyond 3 h. The combination therapies studied are anti-thrombotic (reteplase) and fibrinolytic (abciximab) provided faster, consistent and sustained reperfusion (Broussalis et al., 2012). Treatment with neuroprotective strategy (hypothermia) with thrombolytic (tPA) agent was promising. Neuroprotective stabilizes threatened tissue and extends the time window for the subsequent administration of reperfusion agent. The other neuroprotective strategies used in ischemia are glutamate receptor antagonist, calcium channel inhibitors and antioxidants (Mestre et al.,

2013; Molina and Saver, 2005). Administering agents that are vasoprotective (matrix metalloproteinase inhibitor batimastat) along with tPA may improve recanalization rates and improve the permissible time window for reperfusion therapy by enhancing the benefit/risk ratio in stroke as well as myocardial infarction incidences (Molina and Saver, 2005).

3.1.3. Sonothrombolysis

Although tPA is the most favored option in the management of ischemic stroke, its transportation and delivery to the site of thrombus are crucial to achieving desirable outcomes. Recent clinical evidence has demonstrated the ability of ultrasound technique to enhance enzymatic thrombolysis (Teng et al., 2017; Chen et al., 2014). Timely application of optimum frequency of ultrasound helps in transporting tPA into the thrombus and promoting the opening, cleavage of the fibrin polymers (Marczin et al., 2003).

Most of the strategies discussed above are aimed at providing the prompt and complete reperfusion of the severely ischemic tissue. Reperfusion injury is the paradox of recanalization of obstructed arteries resulting in major cellular dysfunctions (Aronowski et al., 1997). The recent research in stroke highlighted the implication of sudden reperfusion-induced cellular damage and hence the emphasis, in drug development for stroke treatment, is on understanding mechanisms of cell injury due to stroke and designing strategies to effectively counter reperfusion injury (Cuzzocrea et al., 2001).

In the last two decades, the research on reperfusion injury is revolving around the concept of oxidative stress management and the restoration of the metabolic balance of the cells prone to injury (Marczin et al., 2003; Cuzzocrea et al., 2001). Various herbs (Thiyagarajan and Sharma, 2004; Du and Ko, 2006), hormones (Zhai et al., 2000; Zhao et al., 2015) and chemical agents (Dong et al., 2015) were experimentally evaluated for its effect on reperfusion injury. Few of these agents were found to be effective but none of these could be taken to the next step of developing an ideal therapy for the reperfusion injury.

4. CROSSTALK BETWEEN OXIDATIVE STRESS AND CELLULAR INJURY

The impact of reperfusion injury related to ischemic myocardial and cerebral conditions is enormous and this enormity of the cerebrovascular and cardiovascular diseases has driven the scientific world in the pursuit of the ideal therapy or strategy to restrict the inflicted damage (Saposnik et al., 2009). Coronary heart diseases and coronary heart diseases take a heavy toll on the population of lower and middle-income group countries. Almost 75% of the CVD burden in the form of mortality and disability of the productive human resource is bore by this group of countries (Alves et al., 2016).

The scarcity of data makes more difficult to analyze the actual extent of these diseases in the developing countries. The major reason for the slow and agonizing progress towards developing an intervention to treat these pathological complications is the absence of a strategy for secondary prevention in CVD and CHD (Alves et al., 2016; Saposnik et al., 2009). The intriguing mechanisms of stroke have always been a quagmire for researchers as well as clinicians. Hence, the lack of therapeutic response to interventions targeting a single mechanism has warranted a study design that analyzes the approach of a combination of various pharmacologically active drugs. This approach gives us the liberty to study the effect

of multiple active drugs acting independently or synergistically in the combination to target more than one underlying mechanism in stroke or reperfusion injury (D'Ascenzo, 2015).

To start with, the extensive review of the mechanisms of injury involved in cerebral ischemia/reperfusion injury was carried out. Various factors assisting the ischemic damage and ensuing disability are discussed in detail while diagrammatic representation provides a view of ROS generation as shown in Figure 1.

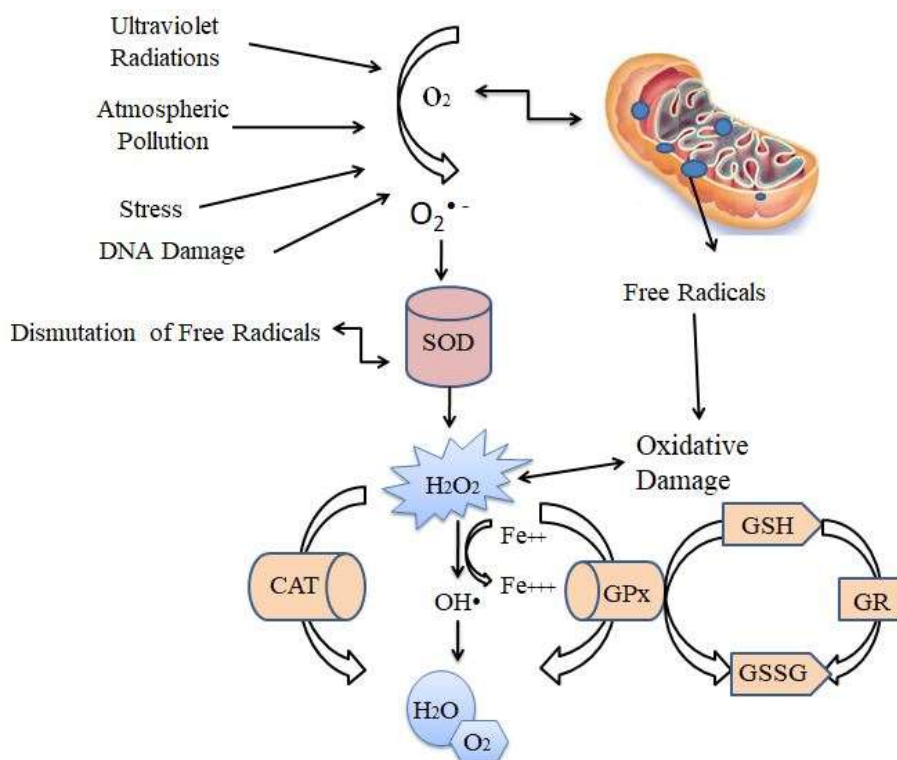


Figure 1. Generation and metabolism of reactive oxygen species. Superoxide radical ($O_2^{\cdot-}$) is produced by ultraviolet radiations, stress, DNA damage, and atmospheric pollution. Superoxide is converted by superoxide dismutase (SOD) to H_2O_2 , which in turn is reduced to water by catalase and glutathione peroxidases (GPx). In the presence of Fenton reaction (iron and hydrogen peroxide are capable of oxidizing a wide range of substrates), H_2O_2 can undergo spontaneous conversion to hydroxyl radical ($OH^{\cdot}$), which is extremely reactive. GPx neutralizes hydrogen peroxide by taking hydrogen from GSH molecules resulting in two H_2O (water) and one GSSG. GPx oxidizes GSH to GSSH with the help of GR. SOD, Superoxide dismutase; CAT, Catalase; GPx, Glutathione peroxidase; H_2O_2 , Hydrogen peroxide; GSH, Glutathione; GR, Glutathione reductase; GSSH, Glutathione disulfide.

4.1. Oxidative Stress Induced by ROS Generation in Myocardial Reperfusion Injury

The myocardial injury is accentuated by the generation of free radicals from different sources during the process of reperfusion injury. ROS can be termed as any chemical species that contains one or more unpaired electrons in outer most orbit making the molecule unstable

and highly reactive. Hence it quickly gets coupled with an adjacent molecule by sharing an electron. This conjugation chemically modifies itself and also become excessively reactive owing to the availability of an extra electron. Consequently, the adjacent molecule follows the pattern of this oxidation and kick starts the chain reaction to generate ROS and in turn damage cell structures (Jennings and Steenbergen, 1985). The free radicals are generated in three ways:

1. Homolytic cleavage of the covalent bond
2. Loss of electron from a stable molecule
3. Addition of an electron to a stable molecule

This generation of free radical depends on the stimuli exerted to the cell. The ROS and others are generated by nitrogen termed as reactive nitrogen species (RNS) (Mimic-Oka et al., 1999). ROS can be divided into radicals and nonradicals. Radicals are superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), peroxy radical ($RO_2^{\cdot}$) and alkoxy radical ($RO^{\cdot}$) come in this category. The nonradicals are hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$) and singlet oxygen (1O_2). Nitric oxide (NO) and nitrogen dioxide (NO_2) are two major nitrogen species responsible for cell damage. The nonradicals in this category are nitrous oxide (HNO_2), peroxyxynitrite ($ONOO^{\cdot}$) and alkyl peroxyxynitrites ($ROONO$) (Cuzzocrea et al., 2001).

The initial surge in ROS generation during reperfusion has a unique mechanism and different source of generation than ischemic milieu. However, the exact source of ROS generation is not yet precisely identified but these cellular redox conditions are thought to be responsible for regulation of vital cell pathways (energy metabolism, survival/stress, and inflammatory reactivity). These are reports contesting conventional views about the exact role of ROS in cerebral as well as myocardial reperfusion injury (Marczin et al., 2003).

Generation of free radicals occurs when oxygen gets reduced through univalent pathway, however, the percentage of total oxygen reduction by univalent pathway is just 5%. Generally, the reduction of 95% oxygen is carried out by mitochondrial electron transport using tetravalent reduction pathway to H_2O where no ROS is produced. Superoxide anion is formed when oxygen accepts an electron and establishes equilibrium with protonated form $HO_2^{\cdot}$ which is favored and more reactive especially during ischemic acidosis conditions (Jones et al., 2003). Dismutation (SOD) of O_2 to H_2O_2 is the key to prevent potential oxidative cellular damage by $RO_2^{\cdot}$. Moreover, it is acknowledged that H_2O_2 is only directly toxic only at high concentration which is unlikely to occur under normal conditions. The cells have the endogenous mechanism to neutralize this formed H_2O_2 by catalase and glutathione system. Superoxide formed as a byproduct of the respiratory chain is safely neutralized to water. However, in ischemia/reperfusion condition this delicate balance is destabilized (Lefer and Granger, 2000). As the ischemia intensifies the defensive mechanism of the cells deplete and H_2O_2 become more empowered to generate the destructive hydroxyl radical ($OH^{\cdot}$) (Sugamura and Keaney, 2011). These hydroxyl radicals are highly reactive and are implicated in lipid peroxidation, destruction of protein as well as sulfhydryl bonds of the cellular structures. Similarly, metal ions like iron and copper are responsible for generating free radical. Especially the chelation of metals is considered to be an approach to reduce oxidative stress owing to the vital role played by iron and copper in oxidative stress-induced cellular damage (Jomova and Valko, 2011).

The Haber-Weiss reaction generates $\text{OH}\cdot$ from $\text{O}_2^{\cdot-}$ and H_2O_2 , accelerates the non-enzymatic oxidation of molecules such as epinephrine and glutathione (Jomova and Valko, 2011).



Oxidative stress induced by ROS generation during reperfusion injury is considered as a defining factor for exacerbating the cell injury in ischemia region of any vital organ (Pizzino et al., 2017). As the reintroduction of oxygen to ischemic cells produce oxygen free radicals after sequential reduction of the oxygen molecule, the formation of superoxide anion, hydroxyl radical, and peroxynitrite is envisaged in early first few minutes of reflow (Kalogeris et al., 2016). Other major contributing components in the generation of ROS are enzymes, such as xanthine oxidase, cytochrome oxidase, and cyclooxygenase, and the oxidation of catecholamines.

In addition to free oxygen radicals, reperfusion also increases inflammatory reactivity through activation of neutrophils (Vinten-Johansen, 2004), consequently, this infiltration of neutrophil also serves as stimuli for generation of ROS. Lipid peroxides are a precursor for lipid peroxidation which impairs functions of enzyme system which are bound to membranes (Rubbo et al., 1994). Poly-unsaturated lipids serve as a primary source of lipid peroxides after interaction with highly reactive free radicals. Endothelial dysfunction occurring in excessive oxidative stress can be traced back to improper release as well as the sensitivity of vessels to nitric oxides which may precipitate in microvascular dysfunction (Radi et al., 1991).

4.2. Key Mechanisms Responsible for Cellular Injury in Stroke

Few realms of disease are as devastating as neurological ones, because such damage can destroy our very essence of life. Stroke and following reperfusion injury is a major source of disability and thus much research is being placed on the treatment and prevention neurological well being (Snow, 2016). Various neuroprotective strategies include free radical scavengers, excitotoxicity inhibitors, and calcium channel blockers. Numerous new interventions are being investigated for the prevention of the severe damage caused by the cerebral reperfusion injury (Molina and Saver, 2005). To list, few of these are various hormones, tissue plasminogen activators and antibodies to adhesion molecules.

Pathogenetically, stroke integrates a heterogeneous group of diseases. Maximum incidences of stroke occur due to occlusion of vessels and account for 85% of all strokes, while few cases represent excessive intracerebral bleeding. Herein, emboli formation in arterio-arterial or cardiac origin is responsible for 75% of all cerebral vessel occlusions and resultant ischemic injury (Soler and Ruiz, 2010). In rare cases, approximately 5% cases of stenoses of cerebral arteries infarction may result in massive neurological deficit (Pongmoragot and Saposnik, 2012; Kumral et al., 2012). The reperfusion of ischemic brain brought by various interventions leads to cellular injury. There are numerous murine models for the induction of focal cerebral ischemia which resembles clinical ischemic stroke (Pant, et al., 2012).

Cellular injury in the brain following stroke is a consequence of multifactorial events occurring with a very short span of time. If the resourceful blood supply is not restored within

3 minutes of the recovery window the cells start experiencing energy deficit which generally ends up in cells resorting to apoptosis or even experience sudden necrotic damage (Piper et al., 2004; Kalogeris et al., 2012; Carmichael, 2005). Deficiency of oxygen and glucose to high demand organ like brain potentially reduces its functional ability and accumulates toxic metabolites which even induce structural damage to cells of the brain (Kalogeris et al., 2012; Granger and Kyietvs, 2015).

The duration and extent of deficient blood flow determine the changes and sometimes the reversibility/irreversibility of the damage to affected cells. Consequently, this situation regulates the intensity variables responsible for future changes (Aronowski et al., 1997). Alteration of electron transport and reduced ATP are two vital variables modulating the extent of blood flow reduction while ROS generation is the third one partially dependent on flow rate (Katsura et al., 1993). However, oxidative stress and ROS generation is a complex phenomenon which also includes the role of electron transport and available oxygen in its activation (Dirnagl et al., 1995). Hence it will be omnipresent even if blood flow is minimum, as slight as just above zero. Incidences of ischemia induce specific and extensive changes which result in cell death. The direct inhibition of oxidative phosphorylation is one of the most important activities in this cascade of events which include decreased pH, reduced ATP availability, ROS generation and increased accumulation of Na^+ leading to loss of ATP substrate for the $\text{Na}^+\text{-K}^+$ pump. The significant change in ionic concentration initiates secondary changes which finally result in damage of ischemic cells (Lipton, 1999).

Almost all focal ischemic models which closely resemble clinical settings involve occlusion of one middle cerebral artery (MCAO) (Ginsberg and Busto, 1989). The partial occlusion of blood flow with a nylon filament induces significant gradation of ischemia from the core of the lesion to penumbra which is an area at risk of cell death lying on the outermost boundary. The core and penumbra of the ischemic lesion exhibit different metabolic conditions within the affected ischemic site. Modification of MCAO can be achieved by coating the filament with poly-L-Lysine or silicon and inserting it into the carotid artery to be advanced at the origin of a middle cerebral artery. This technique generally ensures minimum variation in lesion size and blood flow restriction (Guan et al., 2012). With MCA occlusion and sudden reperfusion of the affected region induces large infarct area of 250 mm^3 at 24 h in rats (Guan et al., 2012; Koizumi et al., 1986; Aspey et al., 1998). Transient obstruction of blood flow increases the temperature in the core area up to 1.5°C due to reduced circulation and irreversible damage to hypothalamus as observed in Wistar rats. In addition, ischemia damages the endothelium of small arteries and also weakens muscles (Nishigaya et al., 1991). The core area of ischemic lesion comprises of the lateral portion of the caudate putamen and somatosensory cortex while the penumbra which can be revived consists of the neocortex and medial caudate-putamen (Belayev et al., 1997). The ischemia and reperfusion injury can affect the brain extensively and events involved in this injury revolve around the ionic balance and ROS generation.

5. MAJOR BIOCHEMICAL CHANGES AND CELLULAR MECHANISMS OF CEREBRAL INJURY

Detailed insight and molecular changes in ischemic cells can provide more information about the extent of injury as well as regulates the chances of recovery from initial insult due to severe ischemia in affected cells.

5.1. Ionic and Metabolic Changes in the Core and Penumbra

The biochemical and ionic changes triggered by stroke strikingly different in core and penumbra. These differences in metabolism strongly suggest a different mechanism for cell death in these two regions (Duverger et al., 1988). The infarct and edema in core and penumbra is the result of massive ion homeostasis disruption and the influx of water into cells of affected tissue (Back et al., 1995). The critical window of 1-3 minutes, experiencing rapid anoxic depolarization with a concomitant rise in extracellular K^+ to 70 mM and decrease Ca^{2+} at flow levels entering in a cell that is present in the core are critical for the cellular death (Martin et al., 1994). During the reperfusion period, after 2 h temporary focal ischemia, extracellular K^+ returns to control levels for 6 h before rising slightly or the remainder of a 24-h recirculation period (Gido et al., 1997). Despite this return to near normalcy, the insult produces severe damage in the form of infarct encompassing entire core (Fiolet and Baartscheer, 2000) and further development of tissue edema due to water influx in the cells. These changes account for the osmotic lysis and the lytic cell death is also referred to as necrosis, which is as a hallmark of the core of the infarct (Nagasawa and Kogure, 1989).

However, the limited damage to penumbra or risk of damage to the area receiving collateral blood supply through smaller arteries is due to lack of permanent anoxic depolarization with concomitant ion changes during the transient period (Back et al., 1995). Moreover, the ATP deficit, which generally maintained in this area at 50–70% of normal, do not fall enough to allow the anoxic depolarization. However, some transient depolarization (Ginsberg and Pulsinelli, 1994; Mizoue et al., 2015) actually originates from glutamate release in the infarct core but prompt reperfusion can salvage the area at risk of damage due to infarct.

5.2. Energy Metabolism in Stroke

The ATP levels in core fall to 25% of basal values and remain there between 5 min and 4 h of ischemia during transient ischemia (Folbergrova et al., 1992). The damage is severe even after restoring the ATP level up to two-thirds of the normal levels with 4 h of ischemic period. Whereas the ATP levels in penumbra remain around 50-70% of normal during this ischemic period (Folbergrova et al., 1995). The glucose utilization fluctuates during the early times and 3.5 h of the ischemia. During ischemia, the blood flows in penumbra declines but it is still much higher than that of the core. Glucose utilization in the cells of contention is one of the reliable indicators of the extent of the damage to core or penumbra (Belayev et al., 1997). Even after complete reperfusion, the required levels of ATP cannot be replenished to

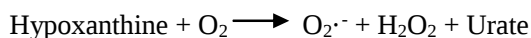
their pre-ischemic values (Piper et al., 2003; Kalogeris et al., 2012). In some cells of penumbra glucose utilization is reduced to well below normal during early reperfusion, and if not restored the cells can later become part of the infarction (Robbins and Swanson, 2014). Reperfusion cannot guarantee prevention of damage despite the rapid recovery of ion homeostasis recovery by reoxygenation. Any single intervention is unable to limit or recover the damage to cells when ischemia lasts for 2-3 h. It will be advisable to administer drugs alone or in combination well before reperfusion to garner the maximum benefits of these interventions in stroke.

5.3. Free Radical Generation in Cerebral Reperfusion Injury

In normal conditions, homeostasis is maintained between free radical generation and elimination of it which is very well tolerated by the cells. However, ischemia-reperfusion injury represents a peculiar sequence of events leading to excessive generation of ROS like superoxide, hydrogen peroxides and hydroxyl radicals. Similarly, the scavenging activity of these ROS is deficient at the massive extent which ultimately leads to dysfunction of the cells in the event of increased oxidative stress (Hammer et al., 1993). Highly reactive peroxynitrite radical, generated from the reaction of superoxide and nitric oxide, can react with virtually any cellular component (carbohydrates amino acids, DNA, and phospholipids) and damage them (Iadecola, 1997).

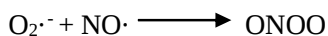
5.3.1. Xanthine/Hypoxanthine Oxidation

Initial stages of ischemia, first 10 minutes after onset, lead to the breakdown of adenine nucleotides which may be metabolized by xanthine oxidase (XO) or xanthine dehydrogenase (XDH). Out of these two, XDH converts adenine nucleotides to nontoxic metabolites but XO generates free radicals which are higher in the brain and other vital organs like liver and intestine (Kinuta et al., 1989). Generation of superoxide radical and hydrogen peroxide is expressed in the given reaction:



5.3.2. Nitric Oxide and Peroxynitrite Generation

Highly reactive peroxynitrite is formed as a result of the reaction between nitric oxide and superoxide which both appears to rise during focal ischemia. Neuronal or endothelial NOS generates NO by activation of Ca^{2+} /calmodulin in most neurons and endothelial cells (Dawson et al., 1994). This Ca^{2+} /calmodulin movement directly activates the NOS enzyme and may also remove a phosphorylation-induced block by activating calcineurin (Yan et al., 2004). During ischemic episode upregulation of both neuronal and inducible NOS initiates reactions resulting in the generation of peroxynitrite:



5.3.3. Eicosanoid Accumulation in Ischemic Brain Regions

Generation of eicosanoids is one more contributing factor for oxidative stress-induced damage. Accumulation of arachidonic acid, via cyclooxygenase (COX) or lipoxygenase (LOX) pathway, serves as a substrate for oxidative metabolism and produces superoxides and hydroxyl radicals (Katsuki and Okuda, 1995; Kontos, 1986; Ikeda and Long, 1990). Break down of phospholipids into Arachidonic acid is increased in ischemia manifold due to the presence of free fatty acid (FFA) which also results in an increased free radical generation (Au et al., 1985; Nakano et al., 1990). During transient ischemia activation of NOS and COX is closely related to ROS generation within 6-24 h period. Similarly, global ischemia also follows an identical path for the free radical generation. The iNOS is present in endothelial cells and invading neutrophils (Nakano et al., 1990), whereas the COX-2 regulated ROS generation in neurons. In the reperfusion phase of injury, COX-2 (prostaglandin H synthase) produces superoxide radicals and iNOS generated NO spearheads the destruction of affected cells. However different cells do have a mode of activation of NOS and COX enzymes (Ahmad et al., 2009; Beckman, 1994).

5.3.4. Ischemia-Induced Alteration in Mitochondrial Function

Free radicals are generated at a brisk pace by the organelles in mitochondria. When electron carried becomes highly reduced, as much as 2% of the total electrons undergo single-electron reduction of oxygen molecule ultimately forming superoxide (Freeman and Crapo, 1982). These reactions very prominently occur in focal ischemia. Accumulation of Ca^{2+} and opening of mitochondrial permeability transition pore (MPT) at some stage of ischemia assist in the generation of free radicals.

5.3.5. Role of Monoamine Accumulation

Monoamine oxidase metabolizes accumulated catecholamines to H_2O_2 within first 5 minutes of ischemia. Superoxide and NO together produce peroxynitrite which mediates much of its toxicity (Birben et al., 2012). Ischemia also brings down the pH of the cell which favors the generation of $\text{OH}^\cdot$ (Birben et al., 2012; Dirnagl et al., 1999) and also by free iron (Sun et al., 2018), as shown below in the reaction generally termed as Fenton reaction:



5.4. pH Changes during Stroke

Severe impairment of blood flow during cerebral ischemia leads to a decrease in intracellular pH (pHi) by 0.5-1 unit. Interestingly pHi at core falls to 6.6, 6.2, and 6.1 when blood flow is reduced to 25% at 10 min and to 10% by 3 and 4 h respectively. However, penumbra at the same time (3h after ischemia) had no significant change in pHi even after a 40% reduction in blood flow (Meyer et al., 1986). Cerebral damage could be exacerbated by this increased acidity due to change in the rate of ROS generation and hence the pH profile during reperfusion is a vital parameter as well as a major contributing factor. Early dip in pHi is associated with immediate blockade of oxidative phosphorylation and the glycolytic flux (Lemasters et al., 1996). Deficient ATP supply also activates glycolysis stemming leading to

increased lactate concentration as blood glucose increased and fall in pH_i (Banani et al., 2000). These events during focal termed as lactate–acidosis-hypothesis is frequently cited in order to explain the glucose paradox of cerebral ischemia and following cellular injury. The paradox is that there is experimental evidence that high supply of glucose, the most important source of energy of the brain, in cerebral ischemia surprisingly induces more damage instead of limiting it as would have expected due to replenishment of ATP during reperfusion. However, this view of paradox is contested by another school of thought (Payne et al., 2003).

5.5. Anoxic Depolarisation and Na⁺-K⁺ Pump in Cerebral Reperfusion Injury

Anoxic depolarization is the unique feature of anoxia/ischemia *in vivo*, this anoxic depolarisation is observed especially in the core region and not in the penumbra (Chen et al., 1993). The significant extracellular negative direct-current shift (10-30 mV) in cell bodies of the ischemic tissue is often accompanied by the precipitous decrease in extracellular Na⁺, Cl⁻ and Ca²⁺ and considered as a characteristic feature of this phenomenon. Anoxic depolarization needs ATP for its initiation and the condition like hypoglycemia where the rate of ATP depletion is increased in hippocampal slices shortens the time to anoxic depolarization (Lipton and Whittingham, 1984). The average value of ATP is 40% at the time of anoxic depolarization and the depolarization occurs within 5 sec of the time at which ATP suffered a precipitous fall (60-90 Sec). This anoxic depolarization is due to Na⁺-K⁺ pump inhibition as it is essentially independent of extracellular Ca⁺ but produces rapid changes in ion concentrations (Nishizawa, 2001). It is still to establish that the pump inhibition is indeed the basis for the anoxic depolarization but the shift in K⁺ could be responsible for the majority of the depolarization (Ferrazzano et al., 2011) and indicates that the neuronal damage is strongly correlated with depolarization (Kaminogo et al., 1998).

5.6. Inflammation in Reperfusion Injury

As discussed earlier iNOS and COX-2 enzyme do regulate inflammatory reactivity in cerebral ischemia but the role of cytokines generated from activated leukocytes play a crucial role in adhesion and transmigration in brain parenchyma. Within a few hours of onset of ischemia, the expression of adhesion molecules at the vascular endothelium and circulating leukocytes induces stroke-induced brain inflammation (Ahmad et al., 2009). Neutrophil granulocytes accumulation in the cerebral microvessels of the penumbra disrupts microcirculation. Similarly, activated leukocytes (granulocytes, monocyte/macrophages, lymphocytes) neurons and glial cells (astrocytes, microglia) produce cytokines and chemokine. TNF- α , IL-1, and IL-6 are proinflammatory, play an important role as mediators of the inflammatory phase (Del et al., 2000), while IL-1 β acts as a substrate for free radical generation and inflammation. Anti-inflammatory cytokines like TGF- β 1 and IL-10 downregulates inflammation and have a protective effect in the context of cerebral ischemia (Bogdan et al., 1992). The iNOS and especially COX-2, by means of the production of free oxygen radicals as well as prostanoids, have a more detrimental effect on the penumbral tissue (Del et al., 2000).

5.7. Phospholipid Metabolism

Phospholipids serve as a major tool for the execution of membrane function and signaling capabilities due to the presence of phosphoinositides (Zhang and Sun, 1995). During ischemic episodes, the breakdown of phospholipids increases the levels of FFA and potentially enhances the rate of ROS generation. The reperfusion, in turn, activates arachidonic acid and result in greater free radical formation. This upregulated arachidonic acid and FFA concentrations and observed in CA1 for more than 1 day after 5 min of ischemia (Abe et al. 1991). Total FFA was increased 4- to 10-fold between the first 15 min and the end of 60 min of MCA occlusion (Zhang and Sun, 1995). The concentration of FFA is dependent on the duration of the ischemic period and the onset of reperfusion.

Metabolism of phospholipids is coupled with large Ca^{2+} influx and energy failures. In addition to energy failure, activation COX and free radical generation during cerebral ischemia present a conducive environment to initiate apoptosis (Rao et al., 1999). It also activates the platelet activating factor which is also responsible for the explicit increase in Ca^{2+} in neurons. The membrane damage induced by this phospholipids break down is of importance in an organelle other than plasmalemma (e.g., mitochondria). Even the loss in phospholipids content is small in the nonmitochondrial membrane may be small but the studies emphasize the importance of membrane damage (Fukai et al., 2011).

5.8. Permeability of Blood-Brain Barrier in Stroke

The integrity of the blood-brain barrier (BBB) depends on the interaction of the cellular matrix, which is composed of endothelial cells and astrocytes. Because of cerebral ischemia, this cellular matrix and the intercellular signal exchange is damaged. Proteases, especially matrix-metalloproteases (MMPs) play an important role in the disruption of the basic fabric of BBB. Upregulation of MMP-9 in endothelial cells during the first 24 h of permanent ischemia, is a result of cytokines production (Gottlieb, 2011) while extracellular edema development is due to activation of Na^+ transport across the BBB (Simard et al., 2007). Inflammatory reactivity increases temperature and as BBB is highly sensitive to temperature variation and this temperature sensitive nature of BBB causes extensive brain damage to endothelial tight junctions. Once the permeability of BBB is increased the transfer of leukocyte recruitment mediators across, creates havoc with respect to cellular damage during ischemia in a rat model of stroke (Venkat et al., 2017).

5.9. Apoptosis

Although the majority of cell death stroke occurs due to necrosis but the role of apoptosis cannot be ignored (Buja et al., 1993; James, 1994). Apoptosis is programmed cell death in the event of deficient energy still aerobic state of metabolism. Programmed cell death proceeds in a genetically programmed series of biochemical and morphological steps designed to avoid the indiscriminate release of cytosolic contents and the ensuing inflammatory response (Martin et al., 1994). The salient features of this type of cell death are chromatin condensation, endonuclease fragmentation of internucleosomal DNA to multiples of 180 to

200 base pair fragments, and cell fragmentation into small membrane-bound vesicles. On the other hand, necrosis, a catastrophic breakdown of cellular homeostasis occurs if oxygen supply crosses the minimum required level. It was however observed that apoptosis is an actively regulated process of cellular self-destruction, which requires energy and de novo gene expression, and which is directed by an inborn genetic program. The final result of this program is the fragmentation of nuclear DNA into typical nucleosomal ladder (Majno and Joris, 1995; Yuan et al., 2016).

Apoptosis, a programmed cell death takes place in a similar time frame as inflammation and involves caspase enzymes resulting in the development of characteristic histological features of apoptosis are membrane-blebbing, chromatin condensation, shrunken cytoplasm with intact organelles, apoptotic bodies in destructed cells (Majno and Joris, 1995; He and Simon, 2013).

Caspases are a unique class of aspartate-specific proteases (McIlwain et al., 2013), which targets nuclear proteins; proteins involved in signal transduction, and cytoskeletal targets (Yuan et al., 2016; Oliver and Vallette, 2005). Internucleosomal DNA fragmentation requires the prior cleavage of a cytoplasmic inhibitor of the apoptosis-specific endonuclease (inhibitor of caspase-activated DNase, DNA fragmentation factor) (Ktazumi and Tsukahara, 2011) and TNF- α mediated caspase activation (Teringova and Tousek, 2017; Kichev et al., 2014). Multiple isoforms of caspase especially, caspase 3 has a central part in the apoptotic signaling cascade, not only in cerebral ischemia (Friedlander, 2003).

DNA-repair enzymes like poly (ADP-ribose) polymerase (PARP) and DNA-dependent protein kinases (DNA-PK) are the substrates that are metabolized by caspase-3 (Friedlander, 2003). PARP identifies single strand disruption and mediate DNA-repair through the generation of ADP-ribose polymers. However, PARP reduces the energy pool of the cell because it consumes ATP. Although partially controversial, the use of ATP by PARP is considered cytotoxic (Yu et al., 2002). The cleavage of the inhibitor of caspase-activated DNase (ICAD) is of particular importance, as caspase-activated DNase (CAD) is activated by this mechanism. CAD leads to the characteristic DNA-laddering. Besides the destruction genetic information, structural proteins (e.g., laminin, actin, gelsolin) that are essential for the integrity of the nucleus and the cell are cleaved by caspases (Cao et al., 2001; Niizuma et al., 2010).

Antioxidants reduce the extent and rate of apoptosis, hence oxidative stress is expected to constitute an important intermediary step in signaling cascade of apoptosis (Ludwig et al., 1996). Apoptosis can be prevented by proteins or survival factors like interleukin-3, nerve growth factor (NGF), insulin-like growth factor-1 (IGF-1), or platelet-derived growth factor. Many neuroprotective agents like erythropoietin activate the PI 3K/Akt-pathway, plays an essential role in this neuroprotective pathway (Hernandez et al., 2017). Chromatin condensation and margination that result in a half-moon or horseshoe appearance of the nucleus are typical features of apoptotic cell death. The most commonly used techniques to detect apoptosis are based on the fact that during apoptosis the genomic DNA is cleaved within internucleosomal DNA segments by an endonuclease selectively activated during apoptosis (Lu et al., 2005).

6. MARKERS OF THE ISCHEMIA/REPERFUSION INJURY

Various mediators and their interrelated mechanisms accentuate the reperfusion injury. In the last few decades, the need for early prognosis of the injury was considered as a vital prerequisite for the treatment and the management of the reperfusion injury (Sinning et al., 2017; Kumral et al., 2012). The research was focused on the development of new markers of the injury for a proper clinical approach to avoid excessive damage and further complications. Few of the bio-indicators of the reperfusion injury are discussed in this section.

6.1. Oxidative Stress/Antioxidant Biomarkers

Oxidative stress caused by the production of free radicals is postulated as one of the earliest mechanisms for the damage to the tissue in the reperfusion injury (Robbins and Swanson, 2014). The generation of various ROS can be assessed by the measurement of endogenous antioxidant enzymes and the level of reduced glutathione. Lipid peroxidation during the injury is also of great importance to predict the damage caused by oxidative stress (Nakano et al., 1990).

6.1.1. Superoxide Dismutase

Oxygen, under normal conditions, is reduced to water (H_2O) by mitochondrial electron transport which reduces 95% of O_2 by tetravalent reduction to H_2O without any free radical intermediates (Sinning et al., 2017) while remaining 5% of oxygen is reduced via the univalent pathway ending up with free radicals production. Superoxide anion is generated after the acceptance of an electron by oxygen. Ischemia-induced acidosis alters the equilibrium of superoxide with its protonated form, $HO_2^\bullet$ and becomes more reactive. $HO_2^\bullet$ is capable of oxidative injury to fatty acids and cell membranes, which is presented within the cell by dismutation (primarily by superoxide dismutase) to hydrogen peroxide (H_2O_2). Under normal conditions, H_2O_2 does not occur in cells, which at higher concentrations is directly toxic to the cells. Cells do convert H_2O_2 to H_2O via catalase or glutathione system ensuring safe metabolism of superoxide, a byproduct of respiration to a humble water molecule (Dunning et al., 2013).

It is observed that a significant decrease in antioxidant enzymes especially superoxide dismutase (SOD) due to ischemic injury leaves affected tissue extremely vulnerable to oxidative stress-induced damage (Fukai and Ushio-Fukai, 2011). Superoxide is one of the most reactive ROS leading to oxidative stress and is generated at various stages of reperfusion following ischemia. SOD is an endogenous ROS scavenging enzyme which is subdivided based on the location where it is highly expressed. In the cytosolic and lysosomal fractions as well as in the mitochondrial intermembrane spaces has a maximum expression of CU/Zn-SOD (cytosolic or SOD1) (Okado and Fridovich, 2001). Similarly, inflammatory cytokines like $TNF-\alpha$, interleukins (IL-1 and 6) and interferon- γ (INF- γ) induce expression of MnSOD (mitochondrial or SOD2) in the mitochondrial matrix. However, overexpression of MnSOD has a protective effect on tissue against reperfusion injury (Ighodaro and Akinloye, 2001). In addition to these isoforms of SOD, brain exhibit extracellular SOD (EC-SOD also termed as

SOD3), a tetrameric protein secreted in extracellular compartment and is unregulated by iNOS after activation of NF κ B (Petersen et al., 2007; Qin et al., 2008).

Thus from all these data, it is clear that SOD enzyme has a critical pathophysiological role in the oxidative stress-induced damage and the mimetic of the SOD may have the clinical importance to restrict the injury.

6.1.2. Activity of Catalase

Hydrogen peroxide is produced by dismutation of superoxide by SOD. This hydrogen peroxide is moderately reactive and has the ability to freely cross lipid bi-layer of the cell (Lafon-Cazal et al., 1993). Hydrogen peroxide is converted to water, however, the intermediate hydroxyl radical formed is more reactive than hydrogen peroxide which is metabolized through the iron-catalyzed Haber–Weiss reaction (superoxide-driven Fenton chemistry) (Zweier and Talukder, 2006). Elimination of hydrogen peroxide is therefore critical to the efficacy of SOD in reducing oxidative stress. Catalase (CAT) and glutathione peroxidase (GPx) serve this purpose (Day, 2009). CAT is mainly localized to peroxisomes.

Thus catalase metabolizes H₂O₂, prevents possible lipid peroxidation. NADPH has a vital role in CAT activity during oxidative stress. NADPH assists CAT by preserving it from excessive H₂O₂ inactivation and provides a source of GPx during stress (Ighodaro and Akinloye, 2017).

6.1.3. Glutathione Depletion and Role of Glutathione Peroxidases

Glutathione is a tripeptide (g-L-glutamyl-L-cysteinyl-glycine) that is the reductant for glutathione peroxidase. This glutathione (GSH) which is made up of cysteine sulfhydryl group is vital for defense against oxidative injury. During ischemic insult, oxidation of the cysteine sulfhydryl group joins two GSH molecules with a disulfide bridge to form glutathione disulfide (GSSG). This oxidized metabolite is restricted as well as recovery of total GSH is catalyzed by NADPH dependent glutathione reductase. The ratio of GSH/GSSG determines the status of oxidative damage, higher the GSH/GSSG ratio smaller is the oxidative damage (Namba et al., 2001). Vital organs like the brain and heart maintain a high ratio of GSH/GSSG which ensures maximum antioxidant defenses in the event oxidative stress in the situations of ischemia and ensuing reperfusion. Even though the GSH/GSSG ratio is key to prevention of damage to the brain but severe depletion of GSH following ischemia/reperfusion may take up to 7 h to restore pre-ischemic values of GSH/GSSG ratio (Warner et al., 2004; Ozer et al., 2005; Namba et al., 2001; Dhalla et al., 2000). Principle consequence of GSH loss during oxidative stress in the brain is the formation of protein-glutathione mixed disulfides (PrSSG) and loss of protein thiols (Purucker et al., 1991). The loss of GSH and formation of PrSSG in the brain results in dysfunction of various membrane functions in cells, such as inhibition of Na⁺K⁺-ATPase activity and mitochondrial enzyme activities (Nakamura et al. 2000).

In this reaction reduced glutathione (GSH) is used as a co-substrate to metabolize H₂O₂, resulting in H₂O and oxidized glutathione (GSSG). Oxidized glutathione (GSSG) can be reduced back to GSH by the enzyme GSH reductase (GR), a reaction requiring NADPH regenerated by glucose 6-phosphate dehydrogenase (G6PDH). The capacity to recycle GSH makes the GSH cycle a pivotal antioxidant defense mechanism for cells and prevent the depletion of cellular thiols (Ighodaro and Akinloye, 2017; Day, 2009).

Glutathione reductase which removes GSSG recycles GSH and makes it available for reuse to avoid the stress injury. The individuals with GSH deficiency may well experience excessive oxidative injury as RBCs hemolysis and renders the endogenous defense system vulnerable to damage. Glutathione peroxidase overexpression was found to reduce the necrotic and apoptotic cell death in the cerebral injury. Various hydrophilic antioxidants (GSH and ascorbic acid) were protective on the myocardial ischemia-reperfusion injury (Ighodaro et al., 2017).

6.1.4. Lipid Peroxidation as Bio-Indicator

The brain has the liability of more easily peroxidizable fatty acids, which consumes a large quantum of total oxygen consumption. Except for a moderate expression of EC-SOD, brain lacks an enriched defensive antioxidant system (Schonfeld et al., 2013). Arachidonic acid concentration is at the highest level due to an increased flux of Ca^{2+} and ensuing energy failures during cerebral reperfusion injury causing damage to cellular membrane (Hendrickson et al., 1997). Inhibition of oxidative phosphorylation (Filmore et al., 2014), formation of proinflammatory mediators (Wang et al., 2016; Filmore et al., 2014), free radical generation and lipid peroxidation-mediated chain reactions (Sun et al., 2018), and cytotoxicity from lipid peroxidation products are major undesirable effects associated with FFA metabolism. These events may stimulate cell death in the vulnerable tissue, particularly in the penumbra region (Ramana et al., 2013). The extent of lipid peroxidation by any noxious stimuli can be measured by estimation of formed malondialdehyde (Grotto et al., 2009) which serves a reliable indicator of lipid peroxidation are shown in Figure 2.

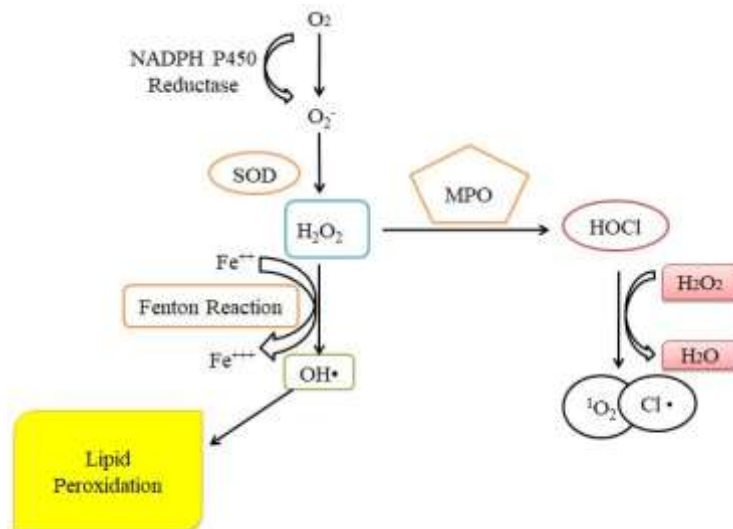


Figure 2. Mechanism of lipid peroxidation. During the metabolism of oxygen, superoxide anion (O_2^-) is formed in presence of NADPH P450 reductase, further superoxide undergoes dismutation to hydrogen peroxide (H_2O_2). Myeloperoxidase (MPO) converts H_2O_2 to hypochlorous acid (HOCl), which is a strong oxidant that acts as a bactericidal agent. During Fenton reaction Fe^{2+} is oxidized to Fe^{3+} and H_2O_2 is converted in the highly reactive hydroxyl radical ($\text{OH}\cdot$), this radical result in lipid peroxidation.

6.2. Creatine Kinase

In search of the markers for ischemia and early predictors of infarct, creatine kinase (CK) was found to be a reliable marker of ischemic injury. Numerous reports considered depletion of CK in cardiac tissue and an index for infarction (Kitzenberg et al., 2016). CK is an important cellular enzyme mediating energy transduction in muscle cells. CK is highly sensitive to oxidation at the exposure of ROS due to the presence of SH groups in cardiac tissue. CK plays a key role in energy production and utilization by ensuring the availability of high energy phosphates throughout the cell (Kjekshus and Sobel, 1970; Saks et al., 1994). The rupture of myocardial cells and destruction of cellular integrity results in leakage of intracellular enzymes like CK from myofibrils after ischemic reperfusion injury. CK activity proved to be a pivotal cardiac marker due to its rapid appearance and marked an increase in serum after acute ischemia (Watts, 1973; Dreyfus et al., 1960). It appears that CK-MB is derived from the necrotic myocardial cells and levels of free release of CK indicate cell death. It was observed that CK release and its correlation with histology and ECG establishes a strong correlation to increased damage to myocardial tissue and is now considered to be a specific diagnostic tool in myocardial infarction (Al-Hadi et al., 2009).

Creatine kinase (MM and BB) is also found in brain tissue and may be elevated in the diseased condition. Mlinarik et al., (1998) studied the rate constants of the CK reaction in the brain after chronic ischemic condition. However, further research is required to establish CK as a reliable marker of cerebral reperfusion injury.

6.3. Myeloperoxidase

Myeloperoxidase (MPO), a polymorphonuclear neutrophil-derived heme protein, is a biomarker index for neutrophil infiltration and inflammatory reactivity. MPO activity is determined by an accumulation of neutrophils which are enhanced during insults like myocardial reperfusion injury is well established (Baldus et al., 2004; Stendahi et al., 1984; Matsuo et al., 1994). Recruitment of polymorphonuclear neutrophils (PMN) attributed to decreased bioavailability of nitric oxide (NO) is responsible for impaired microvascular functions. Whereas MPO facilitates oxidative NO consumption and impairs vascular function in animal models of acute inflammation. Upon activation, PMN not only generates superoxide and H_2O_2 but they also secrete MPO. MPO in the presence of H_2O_2 is anatomically poised to catalytically consume endothelial-derived NO; thereby impairing NO-dependent signaling in smooth muscle cells and subsequent vascular relaxation thus contributes in the endothelial dysfunction (Matsuo et al., 1994; Davies, 2011).

The molecular mechanism MPO mediated toxicity to the cell is halogenation and oxidation. Halogenation involves the covalent binding of the halide co-factor to the target cell or molecule. Among the possible products formed with protein acceptors and iodide as the cofactor are iodotyrosines, iodohistidines and sulfenyl iodides (Baldus et al, 2004). The presence of these chemical configurations near the active site could cause loss of biological activity of the cells. The oxidation may be mediated by such peroxidase system intermediates as halogens, hypohalous acid, chloramines, aldehydes, and singlet molecular oxygen. These MPO components react with extracellular halogen and damage the tissue in the vicinity of the

inflammatory response as the cell-free peroxidase system destroys leukocyte, platelets and other mammalian cells (Davies, 2011).

Increased brain MPO activity (in pericore region) after transient focal ischemia virtually reflects the neutrophil infiltration and this neutrophil infiltration into the ischemic brain is implicated in post-ischemic brain injury (which was evident from the histological observations) (Matsuo et al., 1994). Thus MPO can be considered as a biomarker to indicate the inflammatory response in the reperfusion injury of heart and brain.

6.4. Measurement of Plasma Fibrinogen

Ischemic injury due to obstruction of blood flow could be the result of inappropriate thrombus formation and profound exploration of links of thrombosis and risks of stroke or myocardial infarction suggested crosstalk between plasma fibrinogen and ischemia (Wilhelmsen et al., 1984). A synergistic effect of fibrinogen levels and blood pressure on stroke indicated high plasma fibrinogen is a risk factor for stroke and myocardial infarction (Stone and Thorpe, 1985). Thus, estimation of plasma fibrinogen could be a better diagnostic tool for exploring the thrombogenic potential of this protein in ischemic incidences and providing impetus to develop new strategies for the treatment and management of stroke (Gawaz et al., 2004).

7. PHARMACOLOGICAL STRATEGIES TO PROTECT THE REPERFUSED TISSUE

Maintaining and recovering the reperfused tissue is the foremost goal of the pharmacological interventions carried out during the reperfusion injury (Zheng et al., 2006). The objective of achieving the protection of the ischemic tissue must be:

- Restoration of obstructed blood flow to the ischemic tissue
- Limiting injury due to sudden reperfusion using interventions
- Reduction in intensity of damage due to reperfusion and restoration of cellular functions (Morel et al., 2012).

The goal mentioned to restore normal physiology of the ischemic and reperfused cells can be achieved by various approaches which include:

- Use of agents restricting ischemic damage also considered being anti-ischemic agents
- Ensuring complete restoration using reperfusion interventions
- Exploration and utilization of innate (adaptive) responses (Margaill et al., 2005; Morel et al., 2012).

7.1. Neuroprotection in Cerebral Reperfusion Injury

Injury score to ischemic stroke is highest in prolonging obstruction of blood flow; however, a narrow window of the first hour is available to achieve maximum neuroprotection. The recanalization within the first hour of ischemic onset is the best approach to rescue cells at risk of damage (Belayev et al., 1997). The most obvious target for achieving the goal of survival of cells, prevention of post-stroke complications and recurrence of stroke incidences are molecular mechanisms like glutamate accumulation, aberrant calcium fluxes, free radical formation and lipid peroxidation (Kalogeris et al., 2016).

Recanalisation of the blood vessel can be achieved by anti-thrombolytic, fibrinolytic and anti-platelet drugs which are already discussed in the stroke therapy section of the introduction chapter. Here, the neuroprotective therapy and the new advances in the treatment of stroke and reperfusion injury are mentioned.

7.2. Antioxidants and the Therapy Based on Antioxidant Markers

Oxidative stress which peaks after the reperfusion phase of ischemic tissue is considered to be a major culprit for the instigation and aggravation of the post-ischemic injury. Reperfusion is accompanied by the excessive release of glutamate and extensive infiltration of cells by neutrophil responsible for the generation of ROS (Vinten-Johansen, 2004; Matsuo et al, 1994).

Generated ROS amplify the profound imbalance found in the neurons and astroglial cells of the ischemic core and penumbra. Although tPA is approved for the treatment of stroke, very few treatments are clinically suitable as of now (Snow, 2016). Hence, considering antioxidant as the most promising therapeutic class available for the management as well as prevention of further damage in the ischemic cells enhances the chances of achieving the set goal of the treatment. Antioxidants have a longer therapeutic window than that of other strategies and can be economically viable options for the treatment. Stroke is considered as an outcome associated with a plethora of overlapping etiologies, strategies targeting multiple mechanisms with help of the combination of a various antioxidant could add on to the benefits individual therapy using right dose and correct timing for the administration of antioxidant agents. However, the translation of these antioxidants strategies in clinics still remains a distant dream (Dhalla et al., 2000)

7.2.1. Inhibition of Lipid Peroxidation

Inhibitors of lipid peroxidation are tested using tirilazad, a non-glucocorticoid steroid in first phase II trial (study of tirilazad mesylate in patients with acute ischemic stroke), while phase III studies were started as RANTTAS (randomized trial of tirilazad in acute stroke) (Johnston et al., 2002). The patients from North America were enrolled whereas patients from Europe, Israel, and New Zealand were tested for the parallel trial of the same agent (tirilazad efficacy stroke study). However, the results from most of these trials are not significant in reducing neuronal damage (Margaill et al., 2005).

7.2.2. Inhibition of Xanthine Oxidase

Allopurinol and oxypurinol were scrutinized for their antioxidant potential. Allopurinol is oxidized by xanthine oxidase to oxypurinol, which in turn brings about marked inhibition of xanthine oxidase by binding to the active site of xanthine oxidase inhibition (Nihei et al., 1989). Thus, allopurinol, as well as oxypurinol, can be administered with the same net mechanistic effect. The cerebral edema can be reduced by either of the agents as allopurinol decreases post-ischemic cerebral uric acid while xanthine and conjugated diene concentrations (Marro et al., 1994; Nihei et al., 1989), preserves ATP (Williams et al., 1992) preventing accumulation of fluid in affected cells (Van et al., 1998). The experimental outcomes of allopurinol in rats are mixed and a possible reason for this could be the inability of the agent to scavenge superoxide and hydrogen peroxide generated by other routes.

Many herbal agents found to have significant protective activity in stroke. *Curcuma longa* (Mohanty et al., 2004) and *Ginkgo biloba* (Clark et al., 2001), were studied for its effect on the murine models for stroke and further research is going on these herbs to develop an ideal herbal therapy for stroke.

7.2.3. SOD and Advance SOD Mimetics

The major endogenous SODs, which is studied widely for its effect on the ischemic brain are CuZn SOD, MnSOD and ECSOD (extracellular). Abundant availability of Cu-Zn-SOD and MnSOD in neural tissue had created major interest in the study of the modulation of these enzymes for the prevention of cellular damage (Ighodaro and Akinloye, 2001). Cu-Zn-SOD overexpression reduces ischemic damage resulting from ischemia/reperfusion (Yang et al., 1994). However, neither Cu-Zn-SOD overexpression nor Cu-Zn-SOD targeted deletion alter the outcome from permanent focal ischemia (Fujimura et al., 2001), indicating the requirement of reperfusion for this enzyme to play a role. Some studies highlighted the role of MnSOD in cellular activity after targeted deletion of this enzyme leading to worsening of the outcome from both temporary and permanent middle cerebral artery occlusion (Kim et al., 2002; Murakami et al., 1998). Similarly, over-expression of Cu-Zn-SOD contributed to inhibiting post-ischemic damage. EC-SOD overexpressing mice have increased tolerance to both focal and global cerebral ischemia, while EC-SOD knock-outs exhibit enhanced damage (Sheng et al., 1999). Administration of M40401, a SOD mimetic exhibited beneficial effects in gerbils (Mollace et al., 2003).

7.2.4. Catalase and Glutathione Peroxidase Activities

Catalase and glutathione peroxidase (GPx) are essential for the elimination of hydrogen peroxide generated during reperfusion. Although both enzymes are present in the brain the activity of GPx is sevenfold greater than that of catalase (Ighodaro and Akinloye, 2017). Further, while glutathione peroxidase is present in the cytosol, catalase is localized mainly in peroxisomes. Overexpression of GPx is implicated in significant reduction in necrotic and apoptotic cell death, astrocytic/microglial activation and neutrophil infiltration. Ebselen is a nonselective, synthetic mimetic of glutathione peroxidase (Muller et al., 1984) inhibits protein kinase C, 5-lipoxygenase, cyclooxygenase and NADPH oxidase (Schewe, 1995) is being studied in ongoing clinical trials (Saito et al., 1998; Yamaguchi et al., 1998). The Japanese Ebselen Study Group performed studies on the efficacy of ebselen in ischemic stroke and got approval for the treatment.

The other two antioxidants on the clinical trial at various phases are edaravone, a free radical scavenger, which delays infarct and edema evolution and decreased the mortality in acute stroke patients. However, improvement in functional recovery is not significant. NXY-059 is another free radical scavenger, which is studied in different continents and the results are encouraging. Its primary outcome is overall recovery and recovery of motor function (Green et al., 2003).

7.2.5. Nitric Oxide Synthase Inhibitor

Nitric oxide is synthesized by more than one isoforms of NOS, inhibition of any of these (eNOS, iNOS, and cNOS) affects the ability of NO to induce beneficial or detrimental effects. Pharmacologic eNOS inhibition would be expected to worsen outcome, secondary to cerebral vasoconstriction and reduced blood flow observed in eNOS-deficient mice (Lo et al., 1996) that have worsened ischemic outcomes. In contrast, upregulation of eNOS activity by treatment with 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors (e.g., simvastatin) caused increased intra-ischemic blood flow and reduced infarct size (Amin-Hanjani et al., 2001). Generation of peroxynitrite seems to be the major source of cellular damage and is correlated with upregulation of iNOS (Suzuki et al., 2002) hence inhibition of iNOS exhibited beneficial effects on ischemic injury in the brain.

7.3. Poly (ADP-ribose) Polymerase Inhibitors

Poly (ADP-ribose) polymerase (PARP) is an enzyme imparting a regenerating effect on nucleic acids after degradation of some codons or entire strands (Enders et al., 1997). Poly (ADP-ribose) is synthesized from NAD through a reaction catalyzed by PARP while its degradation is mediated by poly(ADP-ribose) glycohydrolase (PARG). The beneficial effect on that PARP knock-out on cerebral infarct in mice, when compared to wild-type counterparts, highlighted the possible role of PARP in neuroprotection (Eliasson et al., 1997).

DNA damage is a basic trigger for the activation of PARP which serves as a repair mechanism however it also results in NAD and ATP depletion which might potentially exacerbate the ischemic injury. As discussed earlier, peroxynitrite generation and its activity is a principal source of DNA damage (Giovannelli et al., 2002; Mandir et al., 2000). Interestingly, Cu-Zn-SOD overexpressing mice do not exhibit post-ischemic PARP activation suggesting that there is a close correlation between process involved in ROS generation and events leading to disruption of DNA strands or amino acid sequences in each strand. (Effects of pharmacological antioxidants on PARP activation have not been reported. It is warranted that any antioxidant having a major effect on PARP activation needs to be systematically evaluated in ischemic cell death or reperfusion injury. Antagonism of PARP or systemic administration of NAD could be a useful tool to improve ischemic outcomes under sudden reperfusion milieu (Yang et al., 2002).

7.4. Spin Traps Application

The spin trap is a method to capture ROS allowing their detection and quantification. Spin trap approach is first studied in the ischemic brain using salicylate, which reacts with hydroxyl radical to form a relatively stable adduct, 2,3-DHBA (Lapchak and Araujo, 2002). In order to establish this approach or restrict reperfusion injury, nitroxide spin probes, nitron spin traps were developed to capture ROS, allowing detection by electron paramagnetic spectroscopy. Recognizing the potential for nitrones to scavenge ROS, it was considered that these compounds might present therapeutic potential (Marshall et al., 2003). Indeed, administration of the spin trap α -phenyl-N-t-butyl-nitron (PBN) is reported to be protective against both global and focal ischemic insults. Moreover, the second generation spin trap, NXY-059 (disodium 4-[(tert-butylimino)-methyl]benzene-1,3-disulfonate N-oxide) has been found to improve ischemic outcome in primates even after 10 weeks of permanent occlusion of the middle cerebral artery, even when treatment was begun as late as 4 h after onset of ischemia (Marshall et al., 2003). NXY-059 has been shown to maintain Akt activation and inhibit cytochrome c release after ischemia (Yoshimoto et al., 2002).

7.5. Glutamate Antagonists

Glutamate-induced excitotoxicity is implicated in the reperfusion injury damage. Hence the neuroprotective therapy can avail one more avenue of glutamate antagonists. A new glycine-site NMDA receptor antagonist SM-31900 is a potent neuroprotective agent without adverse effects and indicates that it is a promising therapeutic for stroke (Ohtani et al., 2003; Hoque et al., 2016). SM-31900 exhibits nanomolar affinity for this receptor site and selectivity relative to other neurotransmitter receptors and ion channels. In rat models of transient and permanent focal cerebral ischemia, postischemic administration of SM-31900 significantly decreased infarct volume, with potency and efficacy superior to gavestinel, another glycine-site NMDA receptor antagonist. Unlike other NMDA receptor antagonists (dizocilpine, selfotel, eliprodil), neuroprotective doses of SM-31900 were not associated with adverse effects, such as psychotomimetic effects, neuronal vacuolization, renal toxicity or cardiovascular effects. (Prous Science, 2006). YM872, an AMPA receptor antagonist, is a neuroprotective compound that ameliorates the deterioration of the hemodynamic penumbra after focal ischemia (Shimizu et al., 1998).

7.6. Inhibition of Interleukins

Interleukin 1 (IL-1) receptor antagonist, anakinra treatment groups showed neuroprotection, particularly in the cortex, with a significant increase in the frequency of surviving cortical neurons. A nonsignificant reduction in the area of focal infarction in the striatum was also seen in the IL-1 receptor antagonist (IL-1ra)-treated groups (Emsley et al., 2005).

7.7. Matrix Metalloproteinases Inhibitors

Evidence implicates the involvement of matrix metalloproteinases (MMPs) in the disintegration of the vasculature and BBB disruption following cerebral ischemia (Misra et al., 2018). Investigators at University of California at San Diego examined the effects of pharmacological inhibition of MMPs with the relatively nonspecific inhibitor batimastat (BB-94; British Biotech) in a rabbit model of tPA-induced hemorrhage after thromboembolic stroke. This study reported that pretreatment with an MMP inhibitor may improve the safety of tPA in the management of acute stroke, preventing intracerebral hemorrhage, although further studies are necessary (Lopchack et al., 2000).

7.8. Heat Shock Proteins

Hypothalamus, especially CA1 and CA3 and dentate granule cells, exhibited strong expression of HSP70 and HSP72. Penumbra which is considered to be the region of cells with salvageable neurons due to collateral blood supply showed marked ability to produce HSP70 (Weinstein et al., 2004). It has been an intriguing correlation between the expression of HSP and resistance to ischemia in focal ischemia. Activation of the transcription factor NF- κ B, responsible for inflammatory reactivity during ischemia is inhibited by HSP expression in cells under ischemic insult. The 70-kDa HSP is proven to be highly protective and this effect could be attributed to protection against apoptosis, by blocking the downstream actions of caspases on activation of PLA₂, the cell nucleus, and other processes (Li et al. 1993; Sharp et al., 2013).

As established in previous approaches use of single therapeutic agents may show potential promise in animal models, its efficacy in the clinical setup is unconvincing. Consequently, exploration of combined therapeutic approaches in stroke is a need of hour owing to multifaceted etiology of stroke-related cell injury. Herein, it is worth to study various agents and evaluated their effects on reperfusion injury alone or in combination with various hormones. The selection of the agents is based on the antioxidant properties exhibited by the drug may offer promising clues for the management of stroke and other ischemic injuries (Margaill et al., 2005).

8. AGENTS WITH ESTABLISHED ANTIOXIDANT ACTIVITY IN REPERFUSION INJURY

This section highlights some of the agents considered therapeutically viable and are considered for experimental as well as clinical therapeutics.

8.1. Lipoic Acid

Lipoic acid (thioctic acid) is 1,2-dithiolane-3-pentanoic acid, is a molecule which contains two thiol groups and 1,2-dithiolane ring, first isolated from liver samples in the early

1950s. Dihydrolipoic acid and lipoic acid are the natural constituents of biological membranes, acting as mitochondrial lipoamide co-factor of α -ketoacid dehydrogenase (Richard et al., 2011; Panigrahi et al., 1996; Packer et al., 1995). Lipoic acid is highly protein bound by an amide linkage to a lysine residue; this linkage is called as lipoyl group, which plays a lead role in many multienzyme complexes (Packer et al., 1995).

A dual acting α -lipoic acid has the ability to act as an antioxidant in fat- and water-soluble tissues in both its oxidized and reduced forms. Its major advantage seems to be rapid absorption in GIT after oral administration and minimum undesirable changes in other gastrointestinal activity makes lipoic acid a potentially effective therapeutic agent in clinical conditions associated with free radical damage (Cardoso do Vale et al., 2003). Lipoic acid is reported to be an excellent antioxidant in studies and radially taken up by the cells. The exogenous α -lipoic acid is metabolized in the liver to where NADH and NADPH systems play a major role in intracellular conversion. Dihydrolipoic acid, the reduced product of lipoic acid produced in intracellular spaces acts as an antioxidant in extracellular space (Packer et al., 1995; Cardoso do Vale et al., 2003).

Rosenberg and Culik in (1959) discovered the ability of lipoic acid as a potent antioxidant when it exhibited protective effect in both scurvy symptoms in vitamin C-deficient guinea pigs and vitamin E deficiency in rats fed a diet lacking α -tocopherol (Chidlow et al., 2002, Packer et al., 1995; Sige et al., 1978).

Lipoic acid is studied for its antioxidant property in various models of ischemia and reperfusion injury. It found to have an antioxidant effect in cerebral ischemia-reperfusion injury owing to its ability to improve survival and protecting the rat brain against reperfusion injury following cerebral ischemia (Panigrahi et al., 1996). The mitochondria are the main site of antioxidant action of α -lipoic acid. The lipoic acid increases the intracellular glutathione levels; it also has a significant influence on ion Fe^{2+} chelation, nitric oxide and other reactive oxidative species scavenging. S-enantiomer of lipoic acid was more effective than the R-enantiomer when administered 1 hour before ischemia (Cardoso do Vale et al., 2003; Panigrahi et al., 1996). Lipoic acid administration attenuates liver ischemia-reperfusion injury (IRI) via the PI3-kinase/AKT pathway which play a pivotal protective role in IRI. Lipoic acid pretreated organs reduce postischemic activation of NF-kappa B and activating protein-1 (Panigrahi et al., 1996). To addition of its protective antioxidant effects, Haramaki et al., 1993, proposed that lipoic acid improve the cardiac recovery after ischemia, similarly, lipoic acid is reported for its effect on glucose uptake activity (Jacob et al., 1996).

α -lipoic acid is not without adverse effect if the dose and the route of administration are not carefully regulated. Patients exposed to very high dose of lipoic acid suffer adverse effects if thiamine deficiency is ignored. It is advisable to test the patient for thiamine deficiency before administration of lipoic acid (Gal, 1965).

Intraperitoneal administration of high doses of α -lipoic acid intraperitoneally to rats may cause fatal complications which can be averted with the prior administration of thiamine. Researchers are in general agreement that α -lipoic acid is capable of scavenging hydroxyl radicals, hypochlorous acid, and singlet oxygen, but not hydrogen peroxide, peroxy, and superoxide (Freisleben et al., 2000). It prevents DNA strand break up induced by singlet oxygen (Passwater et al., 1995). Lipoic acid is rightly so regarded as a dual antioxidant owing to its pro-oxidant effect at higher doses and very effective antioxidant action at low doses along with its characteristic ability to act as an antioxidant in fat- and water-soluble tissues in both its oxidized and reduced forms.

8.2. Coenzyme Q10

Coenzyme Q10 (CoQ) is also termed to be ubiquinone owing to its omnipresence in almost all cells. A lipid-soluble benzoquinone, COQ, is an essential component for electron transport in oxidative phosphorylation of mitochondria (mitochondrial respiratory chain) (Sunamori et al., 1991). Principally it acts as an electron carrier between the NADH and succinate dehydrogenases and the cytochrome system (Sunamori et al., 1991; Kagan et al., 1998). CoQ functions as an intracellular antioxidant, presumably by preventing both the initiation and propagation of lipid peroxidation (Ernster, 1993; Ernster, 1993).

When the heart experiences oxidative stress, the amount of CoQ decreases which triggers a signal for increased CoQ synthesis (Das and Maulik, 1995 and Crestanello et al., 1996). As it is synthesized by *de novo* synthesis CoQ can be endogenously regenerated and is capable of acting as an antioxidant to reduce oxidative damage during reperfusion (Beyer, 1992; Sugawara et al., 1990). CoQ has a potential antioxidant effect in the brain by reducing lactate concentration, inhibiting lipid peroxidation and improves recovery of brain metabolism in ischemic condition (Matthews et al., 1998; Liang et al., 2017).

Mitochondria are not the only site of action for antioxidant activity of CoQ, any cell exhibiting CoQ is expected to have beneficial effects of its protective actions (Sohal and Forster, 2007) making CoQ an ideal therapeutic agent to investigate in cerebral ischemia-reperfusion injury (Garrido-Maraver et al., 2014).

8.3. Trimetazidine

Trimetazidine, 1-(2,3,4-trimethoxybenzyl)piperazine hydrochloride is a FDA approved an anti-ischemic drug used in myocardial infarction and angina pectoris (Kutala et al., 2006). Trimetazidine (TMZ) exerts its actions without any alteration of hemodynamics of the heart (Lopaschuk et al., 2003). Principally it suppresses mitochondrial 3-ketoacyl-CoA thiolase (3-KAT) thereby reducing the free fatty acid metabolism and balancing the cardiac metabolism. TMZ positively modulates the energy homeostasis of the cells under ischemic stress or cells deficient of the energy substrates (Kantor et al., 2000; Guarnieri et al., 1993). TMZ is reported to preserve the cellular homeostasis by controlling intracellular acidosis and calcium overload in the cells thereby conserving the valuable ATP stores to meet the energy requirements (Mironova et al., 2001). The administration of TMZ is found to have beneficial effects on antioxidant enzymes in the brain by limiting the depletion of GSH (Abdel-Salam et al., 2011).

The pathogenesis of cerebral ischemia and myocardial ischemia leading to an array of critical cellular changes has similarities in a mechanism of cell death. These changes in cellular and mitochondrial levels are responsive to TMZ (Harpey et al., 1989; Kay et al., 1995). Being lipophilic, TMZ crosses the blood-brain barrier (BBB) ensures the availability at the target site providing prominent neuroprotective agent in cerebral ischemia/reperfusion (I/R) injury. The metabolic effects of TMZ during ischemia are attenuation of intracellular acidosis while in reperfusion it was accompanied by an acceleration of the resynthesis of phosphocreatine and a larger reconstitution of the ATP pool. Hence, TMZ has protected the ATP reserves by streamlining the ATP-producing processes, probably at the mitochondrial level (Dezsi, 2016; Dhote and Balaraman, 2008).

9. RECENT DEVELOPMENTS, FUTURE OPPORTUNITIES AND CHALLENGES

In the recent past, the renewed interest in stroke and reperfusion injury has driven numerous investigations of neuroprotective agents using various murine models of stroke. Many of these agents exhibited potent antioxidant activity indicated by marked changes in biomarkers of oxidative stress. The pharmacological activity extends from scavenging of superoxide anions to attenuating the oxidation of lipid peroxides. Many of these antioxidants do have a major effect on inflammation as well as cell damage due to apoptosis and necrosis. A summarized display of the various stages of pharmacological and clinical research has been given in the tabular form (Table 1 and Table 2).

Having said that extensive research is driving the development of new antioxidant agents which may have potent neuroprotective activity in preclinical stages, their translation in clinical therapeutics is still debatable (Gilgun-Sherki et al., 2002; Flores et al., 2014). Looking at the stringent criterion for clinical studies and slow pace of antioxidant-based neurotherapy development, use of the innovative approach to ensure realistic translation of preclinical gains in robust clinical strategies is the need for scientific research.

Table 1. Neuroprotective agents in phases of clinical development.

Neuroprotective Agents	Mechanism of Action	Stage of Clinical Development
Desmoteplase (Broussalis et al., 2012)	Activates plasminogen conversion to the serine protease, plasmin	Phase 2, 3 and 4 ongoing
Tenecteplase (Broussalis et al., 2012)	Selectively converts thrombus-bound plasminogen to plasmin	Phase 2
Anctrod (Broussalis et al., 2012)	Cleaves fibrinogen rather than fibrin	Phase 3
Edaravone (Broussalis et al., 2012)	Increases eNOS and decreases iNOS	Phase 4
Eptifibatide (Mestre et al., 2013)	Reversibly binds to the platelet receptor glycoprotein (GP) IIb/IIIa of human platelets	Phase 1 and phase 2
Ebselen (Azad and Tomar, 2014)	Reduction of ROS	Phase 3
Minocycline (Veltkamp and Gill, 2016)	Reduction of microglial activation reduced NO production, and inhibition of apoptotic cell death	Phase 2
Triiodothyronine (T3) nanoparticle (Mdzinarishvili, 2012)	Non-genomic T3 activation of nitric oxide signaling and vasodilation. Crosses the BBB via a specific transporter called monocarboxylate 8 (MCT8)	—

Table 2. List of various herbal antioxidants investigated using the various model of ischemia/reperfusion injury.

Antioxidant	Stroke Model	Dose and Route	Biomarkers Evaluated
<i>Canna indica</i> roots (Talluria et al., 2018)	BCCA; Male Wistar rats	400-800 mg/kg; PO	SOD, CAT, and MPO
Hexahydro-curcumin (Wicha et al., 2017)	MCAO; Male Wistar rats	20-40 mg/kg; IP	SOD, GSH, and GPx
Grape seed Procyanidin (Kong et al., 2017)	Transient MCAO; male SD rats	50 mg/kg; IP	GPx
Kukoamine A (Liu et al., 2016)	Permanent MCAO; Male SD rats,	5,10, 20 mg/kg; IV	SOD
Nobiletin (Zhanga et al., 2016)	Permanent MCAO; Male SD rats,	25 mg/kg; IP	HO1, SOD, and GSH
<i>Pentapetes phoenicea</i> (Sravanthi et al., 2016)	BCCA; Male Wistar rats	100 mg/kg; PO	SOD, CAT, and GPx
Arjunolic acid (Yaidikar et al., 2015)	MCAO; Wistar rat	10 and 20 mg/kg; PO	SOD, CAT, GSH, and GPx
Pinocembrin (Saad et al., 2015)	BCCA; Male Wistar rats	10 mg/kg; PO	LPO, GSH
Solasodine (Sharma et al., 2014)	BCCA; Male Wistar rats	100 and 200 mg/kg; PO	SOD, CAT, GSH, and LPO
Sesamin (Ahmada et al., 2014)	MCAO; Swiss Albino mice	30 mg/kg; IP	SOD, GSH, and LPO
Calycosin (Guo et al., 2012)	MCAO; Sprague Dawley rat	7.5,15,30 mg/kg; PO	SOD, CAT and GPx
S-Nitrosoglutathione (Khan et al., 2012)	MCAO; Wistar rats	3 micro mol/kg; IP	SIN-1, GSNO
Cymbopogon martini (Buch et al., 2012)	BCCA; Wistar rats	50-100 mg/kg; PO	SOD, CAT, GSH, and GPx
Ginsenoside (Ye et al., 2011)	MCAO; Swiss albino mice	50 mg/kg; IP	SOD, CAT, GSH, and GPx
Shikonin (Wang et al., 2010)	MCAO; male Kunming mice	50, 25, and 12.5mg/kg; PO	SOD, CAT, and GPx
<i>Bacopa monniera</i> (Saraf et al., 2010)	Transient ICA; Wistar rats	120 mg/kg; PO	LPO

BCCA, Bilateral carotid artery occlusion; MCAO, Middle cerebral artery occlusion; GSNO, S-Nitrosoglutathione; SIN-1, 3-Morpholinosydnone imine HO-1, Heme oxygenase-1; ICA, Intracarotid artery occlusion; PO, Per oral; IV, Intravenous; IP, Intraperitoneal

Very often reports of potent neuroprotection promised in preclinical studies by agents having an excellent reduction in infarct size fails to in translating realistic improvement in clinical trials. The reasons for this discrepancy are associated with disagreements in etiology, age, co-morbidities, therapeutic time window, optimum doses to its appropriate route of administration in preclinical and clinical milieu during the stroke (Gilgun-Sherki et al., 2002;

Flores et al., 2014). Classical example reinforcing these limitations is disodium 2,4-disulfophenyl-N-tert-butyltrione (NXY-059).

NXY-059 acts by trapping free radicals during reperfusion and ischemic episode which attributed to its anti-ischemic activity (Bath et al., 2009; Day et al., 2009). NXY-059 exhibited 43% attenuation of infarct volume indicating potent neuroprotection in a stroke model. Similarly, non-human primates (marmosets) model which closely resembles clinical etiology of stroke also confirmed neuroprotection induced by NXY-059. It fulfilled all STAIR criteria which revolves around post-stroke neurological recovery and hence promised a breakthrough in the treatment of stroke. The initial clinical study, Stroke-Acute Ischemic NXY Treatment (SAINT I), encouraged researches as it indicated a significant reduction in disability after 3 months of stroke when patients treated with NXY-059 in combination with thrombolysis (Lees et al., 2006). More elaborate and statistically powerful SAINT II study could not endorse the earlier potent outcomes and pooled data of two studies indicated a lack of functional improvements and benefits after NXY-059 (Diener et al., 2008). Other examples in the list are tirilazad (U74006F), a 21-aminosteroid which scavenges ROS, inhibits lipid peroxidation (Umemura et al., 1994) and edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) (Feng et al., 2011) (117 of SMI). However, edaravone is approved in Japan in spite of limited clinical data for acute ischemic stroke.

These examples emphasize the need for streamlined and aligning preclinical studies with robust clinical study criteria. The existing discrepancies are lack of pharmacokinetic considerations before treatment optimization, neglected co-morbidity status, inappropriate therapeutic time window, missed primary endpoints on efficacy and homogeneity of animals induced with stroke. These discrepancies seem to be just a broad overview of major limitations of current preclinical models to match clinical etiology of stroke and criteria of clinical studies. It has been suggested that preclinical studies can also be phased out on the lines of clinical trials where phase I would aim to discover or investigate pathophysiological mechanisms of a proposed treatment. While phase II pre-should evaluate the efficacy and safety of a drug and phase III must be conducted in the international, multicentre preclinical setup is required to confirm the efficacy of the proposed therapeutic agent. Similarly, the registration of preclinical studies for primary and secondary endpoint and its definitions in the registry, which are regularly followed in clinical trial registration protocol, must be made mandatory for all preclinical studies.

CONCLUSION

There are numerous more promising approaches which are aimed toward neuroprotection as well as cognitive recovery. Despite the pitfalls of current techniques, the translational activities are being refined with continuous enrichment of current scientific know-how of stroke etiology and glaring gaps in transition to clinics. Further innovative concepts and continuous exploration of the novel hypothesis will definitely result in the development of the new drug for the treatment of stroke in the near future.

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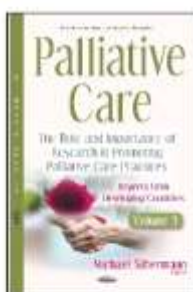
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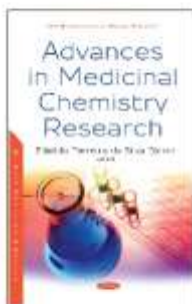
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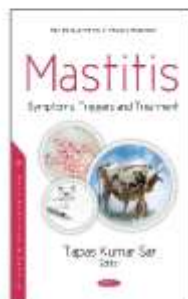
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